PHYSIOLOGICAL REVIEWS
Vol. 80, No. 1, January 2000
Printed in U.S.A.

Anion Transport in Heart

JOSEPH R. HUME, DAYUE DUAN, MEI LIN COLLIER, JUN YAMAZAKI, AND BURTON HOROWITZ

Department of Physiology and Cell Biology, University of Nevada School of Medicine, Reno, Nevada

I. Introduction 31
II. Sarcolemmal Chloride Channels 33
A. Cl^- channels activated by PKA 33
B. Cl^- channels activated by PKC 45
C. Cl^- channels regulated by cell volume 47
D. Cl^- channels activated by cytoplasmic Ca^{2+} 56
E. Cl^- channels activated by purinergic receptors 59
F. Other ClC Cl^- channels 61
III. Phospholemman, P-glycoprotein, and pICln 62
IV. Regulation of Intracellular Chloride Concentration 63
V. Physiological and Clinical Significance of Sarcolemmal Chloride Channels 64
VI. Chloride Channels in Intracellular Membranes 67
A. Sarcoplasmic reticular membranes 67
B. Nuclear membranes 68
C. Mitochondrial membranes 68
VII. Conclusions and Future Directions 68

Hume, Joseph R., Dayue Duan, Mei Lin Collier, Jun Yamazaki, and Burton Horowitz. Anion Transport in Heart. *Physiol. Rev.* 80: 31–81, 2000.—Anion transport proteins in mammalian cells participate in a wide variety of cell and intracellular organelle functions, including regulation of electrical activity, pH, volume, and the transport of osmolites and metabolites, and may even play a role in the control of immunological responses, cell migration, cell proliferation, and differentiation. Although significant progress over the past decade has been achieved in understanding electrogenic and electroneutral anion transport proteins in sarcolemmal and intracellular membranes, information on the molecular nature and physiological significance of many of these proteins, especially in the heart, is incomplete. Functional and molecular studies presently suggest that four primary types of sarcolemmal anion channels are expressed in cardiac cells: channels regulated by protein kinase A (PKA), protein kinase C, and purinergic receptors ($I_{Cl.PKA}$); channels regulated by changes in cell volume ($I_{Cl.vol}$); channels activated by intracellular Ca^{2+} ($I_{Cl.Ca}$); and inwardly rectifying anion channels ($I_{Cl.ir}$). In most animal species, $I_{Cl.PKA}$ is due to expression of a cardiac isoform of the epithelial cystic fibrosis transmembrane conductance regulator Cl^- channel. New molecular candidates responsible for $I_{Cl.vol}$, $I_{Cl.Ca}$, and $I_{Cl.ir}$ (ClC-3, CLCA1, and ClC-2, respectively) have recently been identified and are presently being evaluated. Two isoforms of the band 3 anion exchange protein, originally characterized in erythrocytes, are responsible for Cl^-/HCO_3^- exchange, and at least two members of a large vertebrate family of electroneutral cotransporters (ENCC1 and ENCC3) are responsible for Na^+-dependent Cl^- cotransport in heart. A 223-amino acid protein in the outer mitochondrial membrane of most eukaryotic cells comprises a voltage-dependent anion channel. The molecular entities responsible for other types of electroneutral anion exchange or Cl^- conductances in intracellular membranes of the sarcoplasmic reticulum or nucleus are unknown. Evidence of cardiac expression of up to five additional members of the ClC gene family suggest a rich new variety of molecular candidates that may underlie existing or novel Cl^- channel subtypes in sarcolemmal and intracellular membranes. The application of modern molecular biological and genetic approaches to the study of anion transport proteins during the next decade holds exciting promise for eventually revealing the actual physiological, pathophysiological, and clinical significance of these unique transport processes in cardiac and other mammalian cells.

I. INTRODUCTION

Anion channels in the heart have been the subject of electrophysiological examination for nearly four decades dating back to the original work in 1961 of Hutter and Noble (188) and Carmeliet (43). In the 1970s, there was general agreement that an increase in Cl^- conductance was largely responsible for the initial rapid phase of re-

polarization of the action potential of cardiac Purkinje fibers. However, later studies raised serious doubts about the identity of this Cl^- conductance, and the eventual application of the patch-clamp technique to enzymatically dispersed cardiac cells in the early 1980s relegated Cl^- channels in the heart, like in some other tissues, to a minor and mundane role of membrane "leak." In 1989, though, the demonstration that a time- and voltage-independent anion leak conductance was tightly linked to regulation by the adenylyl cyclase-cAMP-protein kinase A (PKA) pathway (13, 164) provided new impetus for further studies of Cl^- channels in the heart.

During the past decade, an ever-increasing amount of energy has been devoted to the functional and molecular characterization of anion channels as well as transport and exchange proteins in sarcolemmal and internal membranes of cardiac cells and to efforts to reveal their physiological and possible pathophysiological role. A representation of our present understanding of the different types of anion channels as well as transport and exchange proteins found in cardiac sarcolemmal and internal membranes, and some of their intracellular signaling pathways, is illustrated schematically in Figure 1. Initially, six different types of sarcolemmal Cl^- currents were functionally identified in cardiac cells. These included Cl^- currents regulated by the adenylyl cyclase-cAMP-PKA pathway ($I_{Cl.PKA}$), protein kinase C (PKC) ($I_{Cl.PKC}$), cell volume ($I_{Cl.vol}$), cytoplasmic Ca^{2+} ($I_{Cl.Ca}$), purinergic receptors ($I_{Cl.ATP}$) (see Ref. 2 for review), and a basally active Cl^- current ($I_{Cl.b}$). This list of putative sarcolemmal anion channels has been simplified somewhat by new evidence that suggests that $I_{Cl.PKA}$, $I_{Cl.PKC}$, and $I_{Cl.ATP}$ in heart may all be mediated by a cardiac isoform of the epithelial cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channel and evidence that $I_{Cl.b}$ and $I_{Cl.vol}$ may be generated by the same protein. Molecular candidates responsible for $I_{Cl.vol}$ and $I_{Cl.Ca}$ presently include the ClC-3 and CLCA1 gene products, and there is emerging evidence for expression of a new type of sarcolemmal anion channel in some cardiac cells, which generates an inwardly rectifying Cl^- current ($I_{Cl.ir}$) and may be encoded by ClC-2.

In addition to these sarcolemmal anion channels, functional studies have provided evidence for expression of a variety of anion channels in internal membranes as well. These include a PKA-regulated anion channel in the sarcoplamic reticular membrane, two types of anion channels in the nuclear envelope, a voltage-dependent anion channel (VDAC) in the outer mitochondrial membrane, and at least two types of anion channels in the inner mitochondrial membrane that may be related to the inner mitochondrial anion conductance (IMAC) described in flux studies. A variety of sarcolemmal anion cotransporters and exchange proteins are expressed in cardiac cells, which include include Cl^-/HCO_3^- exchange, Na^+-dependent Cl^- transport, K^+-Cl^- cotransport, and a novel Cl^-/OH^- exchanger.

It is becoming increasingly clear that anion channels and transport and exchange proteins in the heart mediate a variety of functions and thus play a potentially important role in cardiac physiology and pathophysiology. Because activation of sarcolemmal anion channels can significantly alter resting membrane potential and the duration of the action potential, these proteins represent novel targets for the development of new antiarrhythmic and anti-ischemic agents. Anion channels and transport proteins in the sarcolemma and internal membranes may be involved in the regulation of cell or organelle Cl^- activity (a_{Cl}), pH, volume homeostasis, and organic osmolyte transport. In many cells, there are also indications that anion transport proteins may play a role in immunological responses, cell migration, proliferation and differentiation, and possibly apoptosis (28, 239). Yet, our present understanding of the physiological significance and clinical relevance of these various anion transport pathways in the heart is incomplete. There is now well-established evidence linking several human genetic diseases to specific anion channel defects (1, 206, 249, 478), but the possible role of defects in anion channels, transporters, or exchangers in the heart to myocardial genetic diseases has not been explored.

The recent molecular identification of some of the proteins responsible for anion transport in the sarcolemma and in internal membranes of cardiac cells heralds a new era for this emerging field. Perhaps one of the greatest impediments to our present understanding of the physiological significance of anion transport proteins has been the lack of available specific pharmacological tools to investigate function. Recent studies are beginning to elucidate well-defined molecular structures for each type of anion channel and transport protein in the heart that should significantly facilitate the development of new Cl^- channel subtype-specific pharmacological tools for future biophysical and functional studies.

The overall aim of this review is to provide a broad overview of progress made over the past decade in the characterization of the molecular, biophysical, and pharmacological properties of anion transport proteins in heart, their species and tissue distribution, and their known or presumed physiological roles. Its content is meant to complement previously published reviews on this subject (2, 136, 162, 186, 189) and to focus on recent new developments, as well as recent controversies, in this rapidly expanding field. Although the major focus of the review is on sarcolemmal anion channels and their signaling pathways in cardiac cells, we also briefly consider the nature of anion channels in internal membranes, and electroneutral sarcolemmal anion transport and exchange proteins, and their physiological roles as well.

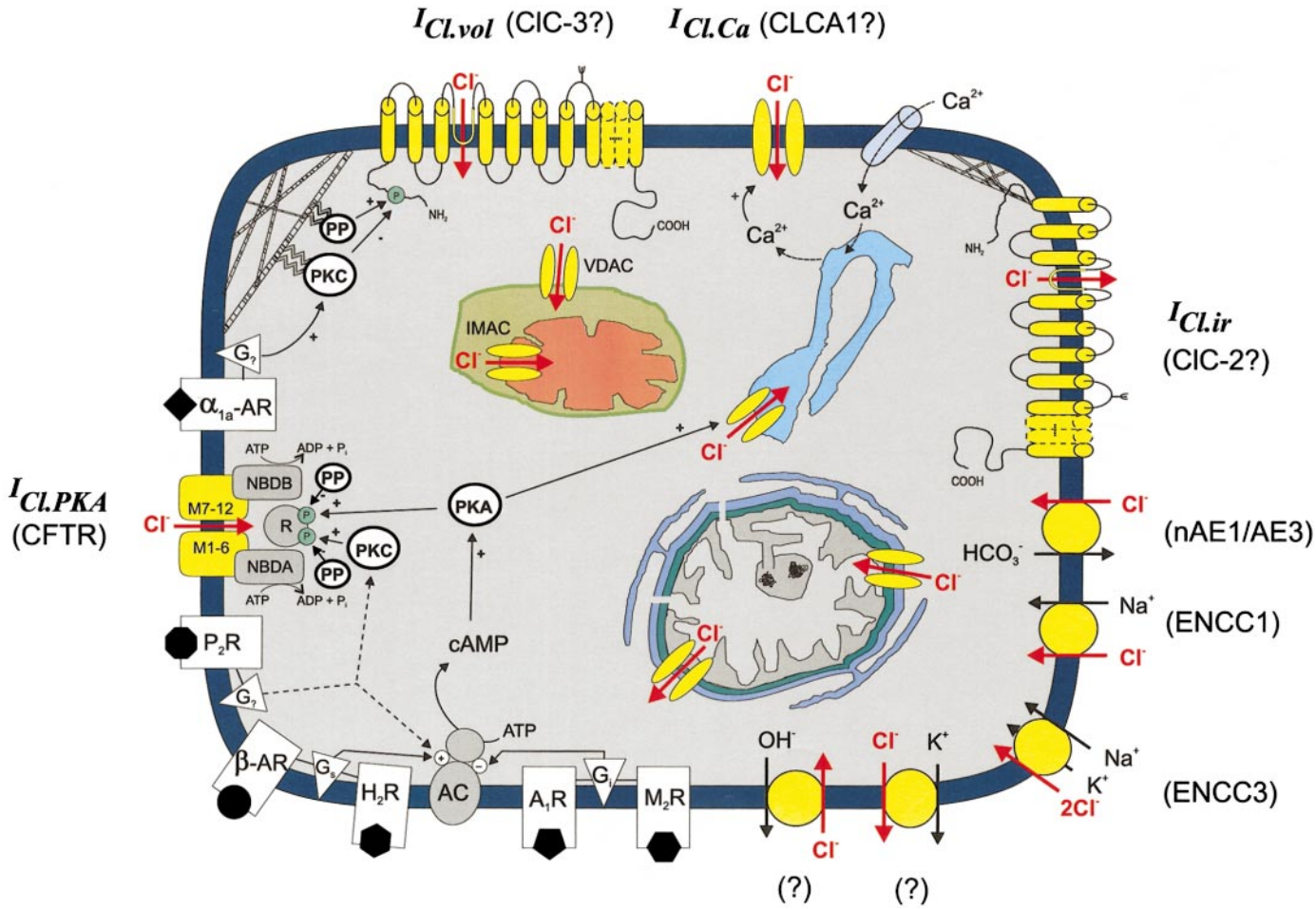


FIG. 1. Schematic representation of cardiac anion channels, transport and exchange proteins, and their intracellular signaling pathways. Anion channels and transport or exchange proteins are indicated in yellow, and their corresponding molecular entities or candidates (?) are indicated in parentheses. $I_{Cl.PKA}$, Cl^- current regulated by adenylyl cyclase-cAMP-protein kinase A pathway; CFTR, cystic fibrosis transmembrane conductance regulator; M1–6, CFTR transmembrane spanning segments 1–6; M7–12, CFTR transmembrane spanning segments 7–12; NBDA, nucleotide binding domain A; NBDB, nucleotide binding domain B; R, regulatory subunit; P, phosphorylation sites for protein kinase A (PKA) and protein kinase C (PKC); PP, serine-threonine protein phosphatases; α_{1a}-AR, α-adrenergic receptor type 1a; $G_?$, unidentified heterotrimeric G protein; $I_{Cl.vol}$, Cl^- current regulated by cell volume; ClC-3, member of voltage-gated ClC Cl^- channel family; $I_{Cl.Ca}$, Cl^- current regulated by intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$); CLCA1, member of a new Ca^{2+}-sensitive Cl^- channel family (CLCA) recently cloned from human intestine (146) and mouse lung (139); $I_{Cl.ir}$, inward rectifying Cl^- current; ClC-2, member of voltage-gated ClC Cl^- channel family; nAE1, truncated form of anion exchange protein 1; AE3, anion exchange protein 3; ENCC1, electroneutral Na^+-Cl^- cotransporter protein 1; ENCC3, electroneutral Na^+-Cl^- cotransporter protein 3; M_2R, muscarinic type II receptor; G_i, heterodimeric inhibitory G protein; A_1R, adenosine type I receptor; AC, adenylyl cyclase; H_2R, histamine type II receptor; G_s, heterodimeric stimulatory G protein; β-AR, β-adrenergic receptor; P_2R, purinergic type 2 receptor; proposed intracellular signaling pathway for purinergic activation of CFTR (96) indicated by dashed arrows; IMAC, inner mitochondrial anion channel; VDAC, voltage-dependent anion channel. [CFTR schematic model from Welsh and Ramsey (476). Membrane topology models for ClC-2 and ClC-3 modified from Jentsch et al. (207) and include a pore-forming region between transmembrane segments 3 and 5 based on Fahlke et al. (110).]

II. SARCOLEMMAL CHLORIDE CHANNELS

A. Cl^- channels activated by PKA

The first evidence for the presence of Cl^- channels activated by PKA ($I_{Cl.PKA}$) in cardiac cells was obtained by two groups in 1989 (13, 164, 165). The macroscopic currents recorded in guinea pig and rabbit ventricular myocytes were selective for Cl^-, exhibited time and voltage independence, and were blocked by anion transport inhibitors. As with Ca^{2+}, K^+, and Na^+ channels in heart, these Cl^- channels were regulated by cAMP-dependent PKA phosphorylation. β-Adrenergic agonists activated the channel subsequent to G protein-mediated stimulation of the cAMP pathway. Soon thereafter, the unitary currents (~13 pS) responsible for this current were identified in

cell-attached membrane patches of guinea pig ventricular myocytes (101). Initially, it was not clear whether or not $I_{Cl.PKA}$ in heart might have a similar molecular basis as $I_{Cl.PKA}$ described in a variety of epithelial cells and known to be encoded by the CFTR gene product (186). Although the macroscopic currents in the two preparations shared a number of similar properties, the unitary currents for $I_{Cl.PKA}$ in heart were linear in symmetric Cl^- and seemed to exhibit a much smaller conductance (102) than the larger outwardly rectifying 25- to 40-pS channels originally associated with CFTR in epithelial cells (475). However, with the successful cloning of the CFTR gene (354, 357), it soon became clear that expression of epithelial CFTR in heterologous cell systems was associated with smaller conductance (4–13 pS) channels. The demonstration that site-directed mutations of lysine residues in the transmembrane domains of CFTR resulted in dramatic changes in anion selectivity of the expressed channels provided strong evidence that CFTR functions as an anion-selective, small-conductance channel, which exhibits a linear current-voltage relationship in symmetric Cl^- (9, 10; see Refs. 127, 129, 353 for reviews). These data along with Northern analysis of mRNA isolated from rabbit (251) and guinea pig ventricle (304) showing hybridization using specific CFTR probes thereafter left little doubt that $I_{Cl.PKA}$ in heart is due to CFTR expression.

The past 6 years have experienced an explosion of new information on the molecular, biophysical, and pharmacological properties of CFTR Cl^- channels and their regulation by intracellular signaling pathways. Several important reviews detailing many of these developments in cardiac (134–136, 162, 187, 189) and epithelial cells (120, 138, 379, 384, 478) have appeared. The focus of this review is to provide *1*) an overview of CFTR Cl^- channel structure and function, regulation, species and tissue distribution, and physiological significance in heart; *2*) an update of new progress made in these areas in the last few years; and *3*) a consideration of some of the controversies that have emerged recently in this field in the heart.

1. Overview of structure and function

The CFTR is composed of 1,480 amino acids, and hydropathy analysis predicts these are organized into two repeating motifs of six transmembrane spanning domains (M1–6, M7–12), two nucleotide binding domains (NBDA and NBDB), and one large regulatory (R) domain that has numerous consensus phosphorylation sites for PKA and PKC. The protein belongs to the ATP-binding cassette (ABC) superfamily of transporters, which are structurally similar in terms of the organization of their transmembrane domains and nucleotide binding domains (170). Over 100 members of this family have been identified including P-glycoprotein (P-gp), which pumps hydrophobic compounds out of cells, and the sulfonylurea receptor (SUR), which combines with inward rectifier K^+ (Kir6.1, Kir6.2) channel subunits to form functional K_{ATP} channels (5, 312). The two transmembrane motifs of ABC proteins are believed to form the pathway for solute transport, while the two nucleotide binding domains are believed to couple ATP hydrolysis to solute transport. Although CFTR seems unique in forming anion-selective channels compared with other members of the ABC superfamily, it may share some characteristic properties of ABC transporters, such as functioning as a pump for the transport of ATP as well as a regulator of other channels, such as outwardly rectifying Cl^- channels (ORCC) and sodium channels (78). However, whether or not CFTR transports ATP remains highly controversial (78, 346, 349, 375).

The contemporary view of CFTR channel function suggests that the highly charged R domain may represent a blocking particle, which in its unphosphorylated form keeps the channel closed, but upon phosphorylation causes channel openings via a conformational change. Phosphorylation of the R domain alone, however, is insufficient to cause channel openings, since hydrolyzable nucleotides are also required, presumably reflecting nucleotide binding to Walker A and B motifs in the NBD, which regulate channel gating properties. Thus phosphorylation of the R domain may promote ATP binding to the two NBD; however, the exact nature of the interactions between the R domain and the NBD remains unclear (78, 379). A variety of studies using site-directed mutagenesis, including scanning-cysteine-accessability analysis, have provided evidence that residues in the first (M1), fifth (M5), sixth (M6), and twelfth (M12) transmembrane spanning domains of CFTR may form part of the ion conduction pathway of the pore region (9, 49, 50, 78, 276, 288, 338, 425). The CFTR channels exhibit a lyotropic permeability sequence that favors weakly hydrated anions: $SCN^- > NO_3^- > Br^- > Cl^- > I^- > F^-$ (259, 490).

Although early studies suggested that the unitary and macroscopic $I_{Cl.PKA}$ in heart exhibited many properties in common with epithelial CFTR channels, including similarities in rectification, anion selectivity, regulation by cAMP-dependent PKA, sensitivity to Cl^- channel blockers, unitary channel properties, and a dependence on hydrolyzable nucleotides for activation (13, 101, 163, 164, 191, 280, 304, 325; see Ref. 136 for review), the first molecular data on the structure of CFTR in heart came in 1993 when the cDNA encoding the 12 transmembrane spanning domains (M1-M12) were cloned and sequenced from rabbit ventricle (182). Comparison of the amino acid sequence of human epithelial CFTR with the deduced sequence from rabbit heart indicated deletion of a 30-amino acid segment in the first cytoplasmic loop of CFTR that corresponds to known locations of intron-exon junctions in human CFTR, suggesting that CFTR is an alternatively spliced (exon 5−) isoform in heart. Outside of the alternatively spliced region, regions M1-M12 of the

heart CFTR isoform displayed >95% identity to human epithelial CFTR. Deletion of exon 5 in the cardiac form was confirmed using Southern analysis of reverse transcription PCR products derived from canine pancreas or rabbit and guinea pig ventricle probed with oligonucleotides corresponding to nucleotide sequences specific for exon 5. The cDNA encoding the complete CFTR exon 5− isoform was subsequently cloned and sequenced from rabbit heart (158) and found to contain ~91% nucleotide sequence homology, outside of the exon 5 region, compared with human epithelial CFTR cDNA, with numerous putative PKA and PKC phosphorylation sites highly conserved in the two isoforms. Although the functional significance of exon 5 remains obscure, this region corresponds to part of the first cytoplasmic loop between M1 and M2 and does contain two putative PKC phosphorylation sites (see Fig. 4*A*). The cDNA encoding the rabbit cardiac exon 5− isoform was expressed in *Xenopus* oocytes and resulted in the appearance of $I_{Cl.PKA}$ that was absent in water-injected control oocytes. This study (158) also provided evidence establishing a direct functional link between expression of CFTR and the endogenous $I_{Cl.PKA}$ in native cells by showing that CFTR antisense oligonucleotides significantly reduced the density of $I_{Cl.PKA}$ in acutely cultured guinea pig ventricular myocytes.

2. Regulation

A) ADENYLYL CYCLASE/PKA. It is now well established that activation of CFTR is a two-step process requiring both PKA phosphorylation of the R domain and binding of ATP to the NBD (138, 379). In cardiac cells, numerous early studies established that $I_{Cl.PKA}$, like I_{Ca} and the delayed rectifier I_K (159, 287), is regulated by the adenylyl cyclase-cAMP-PKA pathway (13, 101, 163–166, 191, 264, 280, 432, 513), and the requirement for hydrolyzable nucleotides was established for $I_{Cl.PKA}$ activation in heart (304) and epithelial CFTR channels (8). However, a mechanistic explanation accounting for the relationship between PKA phosphorylation of the R domain, ATP binding and hydrolysis at the NBD, and the control of CFTR channel gating properties remains elusive. This is due in part to the complicated structure of the protein, which contains at least 10 putative PKA phosphorylation sites (8 in the R domain), difficulties in demonstrating ATPase activity of the NBD biochemically, and a general lack of understanding of the dynamic interactions that may occur between the NBD and the R domain in vivo.

Gadsby and colleagues (190, 192) in a series of revealing studies of CFTR channels in guinea pig ventricular myocytes provided new insights into the relationship between PKA phosphorylation, ATP binding, and hydrolysis and the control of CFTR channel gating. An examination of the dephosphorylation of channels revealed that complete dephosphorylation required both okadaic acid-sensitive [protein phosphatase (PP) 1 and PP2A] as well as okadaic acid-insensitive phosphatases, consistent with an activation (deactivation) scheme involving sequential phosphorylation (dephosphorylation) of the protein (190). The sequential model proposed suggested that the okadaic acid-sensitive phosphatase, PP2A, dephosphorylated partially phosphorylated (P_1) channels, whereas an okadaic acid-insensitive phosphatase dephosphorylated a second phosphorylation site (P_2) on the R domain. Although the exact identity of the okadaic acid-insensitive phosphatase involved was not made, it was postulated to be PP2C, since PP2B was likely to be inactive when intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was buffered to low levels. Although it has proven difficult to definitively implicate PP2C, due to the lack of specific inhibitors, recent studies have shown that the application of recombinant PP2Ca to membrane patches from airway and intestinal epithelial cells (443) or purified PP2C to membrane patches of airway epithelial cells and CFTR transfected Chinese hamster ovary (CHO) cells (271) caused potent deactivation of CFTR channels. Which phosphatases are important seems to be cell type specific, since in some cells alkaline phosphatases (22) or PP2B (114) may also be involved.

Earlier studies had established a link between ATP binding to the NBD and channel gating by showing that mutations in either NBD altered the ability of MgATP to activate CFTR and that similar mutations in NBDA and NBDB were not functionally equivalent, with mutations in NBDA (K464Q and D572N) decreasing the sensitivity to MgATP, while analogous Walker A and B mutations in NBDB (K1250Q and D1370N) increased sensitivity (11, 395). Differential effects of the two NBD on channel gating were also revealed in subsequent studies of the effects of the nonhydrolyzable nucleotide 5′-adenylylimidodiphosphate (AMP-PNP) on CFTR channels in membrane patches from guinea pig ventricular myocytes (192). It was shown that although AMP-PNP was not capable of activating phosphorylated channels in the absence of ATP, fully phosphorylated (but not partially phosphorylated) channels once activated by ATP were significantly stimulated by AMP-PNP due to a marked prolongation of mean open times (see also Ref. 150). These differential effects of AMP-PNP on the two NBD, the demonstration that channels exhibit modal gating behavior, and the observation that open probability (P_o) may correlate with the phosphorylation state of the channels (low P_o for P_1 state, high P_o for P_1P_2 state) (115, 192), led Gadsby and co-workers to propose the model shown in Figure 2 to explain the control of CFTR channel gating by sequential phosphorylation and ATP hydrolysis at the two NBD. In this model, channels exist in one of three phosphorylation states: dephosphorylated (Fig. 2, *left*), partially phosphorylated (Fig. 2, *middle*), or fully phos-

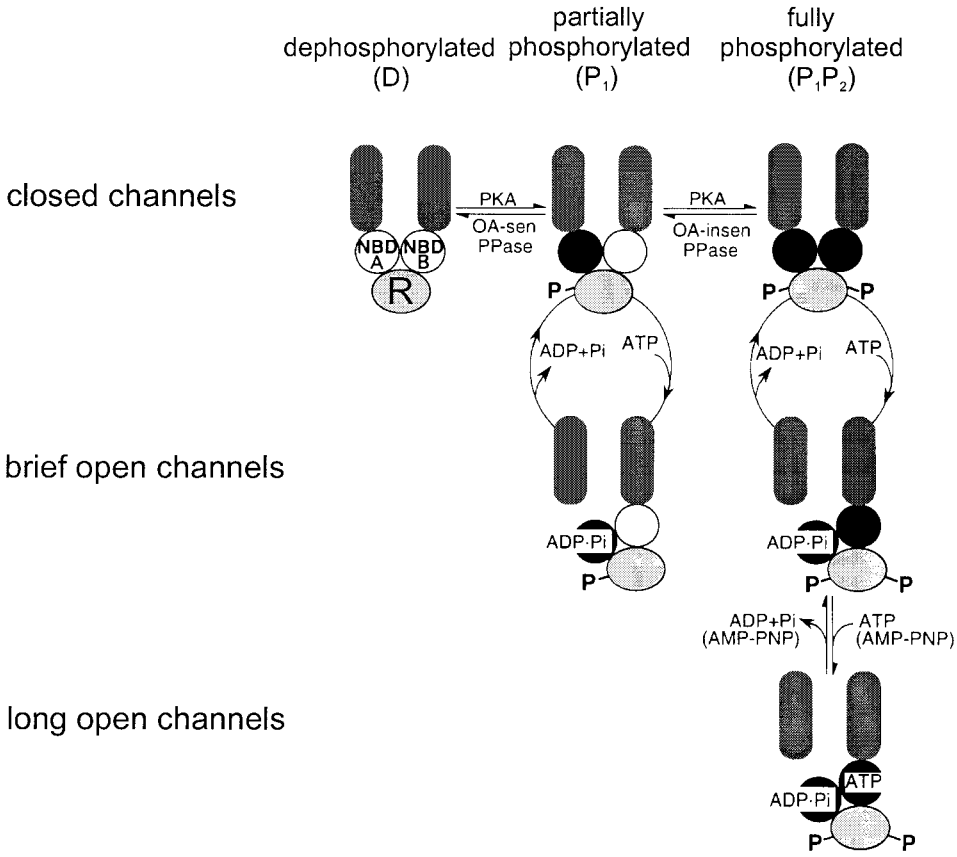


FIG. 2. Schematic model of CFTR regulation by PKA phosphorylation and ATP hydrolysis. Sequential phosphorylation of 2 distinct sites or sets of sites induces a conformation change of the regulatory (R) domain, causing activation of the 2 nucleotide binding domains (NBD-A and NBD-B). The 2 phosphorylation sites are distinguished by their differential sensitivity to okadaic acid. Top row depicts closed channels states that may be dephosphorylated (D), partially phosphorylated (P_1), and fully phosphorylated (P_1P_2). ATP hydrolysis at NBDA is associated with brief channel openings; ATP (or AMP-PNP) interactions with NBDB stabilizes the open channel state, leading to longer channel openings. [Modified from Hwang et al. (192).]

phorylated (Fig. 2, *right*). The phosphorylation state determines the functional state of the NBD and hence channel P_o. For channels partially phosphorylated, ATP hydrolysis at NBDA causes brief channel openings (low P_o), whereas for fully phosphorylated channels, NBDB becomes available and ATP binding at this site stabilizes channel openings leading to enhanced P_o. It is the hydrolysis of ATP at NBDB that controls channel closure. Further evidence in support of this model was obtained in subsequent studies showing that channels partially or fully phosphorylated became locked open for long periods of time when ATP hydrolysis was interrupted by exposure to VO_4 or BeF_3 (17). Mutagenesis of the conserved Walker A lysines in NBDA (K464) and NBDB (K1250) of the CFTR protein subsequently confirmed that the former caused decreases in channel burst frequency, whereas the latter prolonged channel burst duration, suggesting that ATP hydrolysis at NBDA initiates channel bursts, while ATP hydrolysis at NBDB terminates channel bursts (45). Similar conclusions were made based on rate analysis of macroscopic currents associated with wild-type and mutant CFTR channels containing amino acid substitutions in the ATP binding pocket (K464 and K1250) of the two NBD (395, 482). The situation may be even more complex, since a recently revised incremental phosphorylation model suggests an additional, moderately phosphorylated, state (138). Similarities and differences in this model of phosphorylation and ATP hydrolysis between CFTR and another member of the ABC superfamily of transporters, P-gp, have been reviewed (380).

An alternative model of the role of the NBD and ATP hydrolysis in the control of epithelial CFTR channel gating was proposed based on the analysis of prephosphorylated single CFTR Cl^- channels reconstituted into planar lipid bilayers (151). Channels were reported to exhibit two open conductance states (O_1 = 9 pS, O_2 = 10.3 pS), and an analysis of reconstituted channels containing mutations of lysines (K464 and K1250) in the highly conserved P-loop region of NBDA and NBDB (which attenuates ATP hydrolysis in other ABC transporters) supported a central role of ATP binding and hydrolysis in channel gating. However, in this model, NBDB seemed to be most important. Specifically, binding of ATP to NBDB was proposed to control the transition between the closed and O_1 channel states, whereas ATP hydrolysis and Mg^{2+} binding at NBDB was required for channel transitions between the two open states, O_1 and O_2. Surprisingly, mutations in NBDA (K464) produced only small effects on reconstituted channel gating (151) compared with the marked effects on gating observed for NBDA mutant CFTR channels expressed in heterologous expression systems (45, 482). Although it is difficult to reconcile

these apparently disparate results, it is possible that the NBDA mutants examined in the reconstituted channel experiments may not be functionally equivalent to those tested in heterologous systems or that the NBD of reconstituted CFTR channels may not necessarily function in the same way to control channel gating as in native channels. There also is little, if any, evidence suggesting that native CFTR channels exhibit multiple open conductance states, although this seems to be a consistent finding for CFTR channels reconstituted into lipid bilayers (150, 430). It is possible that because of the limited frequency response of the bilayer system, rapid channel gating events may give rise to the appearance of subconductance states (120). Whether or not CFTR channel gating exhibits genuine bursting behavior also has yet to be firmly resolved (120), even though burst analysis is commonly employed to quantitatively assess the functional effects of various channel mutations. Rapid channel closures may reflect block by impermeant anions (195, 258), which under some conditions cause rectification of the macroscopic currents (326). Obviously, a more thorough basic understanding of CFTR channel gating properties will help to eventually delineate the functional role of the NBD in channel gating.

A comparison of the rate-limiting steps for activation of L-type Ca^{2+} channels and $I_{Cl.PKA}$ by β-adrenergic agonists and caged cAMP in native cells reveals interesting differences in the regulation of the two channels by the adenylyl cyclase-cAMP-PKA pathway. Both the stimulation and washout of the effects of isoproterenol on $I_{Cl.PKA}$ were more rapid than on I_{Ca} (175). Activation of I_{Ca} by rapid application of β-adrenergic agonists is associated with an initial latency period, which was not observed after photolysis of caged cAMP, suggesting that the rate-limiting step in the activation of I_{Ca} may be due to a step associated with activation of adenylyl cyclase and accumulation of cAMP (123). Another study (306), which directly compared the activation of I_{Ca} to $I_{Cl.PKA}$ by β-adrenergic agonists and photolysis of caged cAMP, found a similar latency period, suggesting similar reaction steps for activation of adenylyl cyclase and cAMP accumulation for activation of both currents. However, after the initial latency, $I_{Cl.PKA}$ activated with a slow sigmoidal onset, in contrast to I_{Ca} which activated much faster. This slow sigmoidal onset for activation of $I_{Cl.PKA}$ disappeared after partial phosphorylation of the channels by exposure of cells to okadaic acid, suggesting that the rate-limiting step for activation of $I_{Cl.PKA}$ might be due to multiple phosphorylation reactions associated with CFTR. This is consistent with the results of phosphorylation studies of CFTR indicating that multiple serine residues on the R domain are phosphorylated by PKA (48, 335).

B) G PROTEINS. The role of G proteins in coupling β-adrenergic receptors and muscarinic receptors to the regulation of $I_{Cl.PKA}$ in heart was established in early studies. Intracellular GTP was shown to be essential for activation of $I_{Cl.PKA}$ by β-agonists as well as for inhibition by muscarinic agonists. The rundown of $I_{Cl.PKA}$ observed in dialyzed myocytes likely reflects the loss of cellular GTP required to maintain G protein signaling mechanisms (180, 191). Indeed, cellular dialysis with GTP or use of the perforated patch technique greatly prevents rundown of $I_{Cl.PKA}$ (180, 504). The effects of GTP can be attributed to convergence of G_s and G_i on adenylyl cylase, and the evidence that the same G protein-adenylyl cyclase-PKA pathway that regulates I_{Ca} and I_K also regulates $I_{Cl.PKA}$ has been reviewed (136). There is recent data suggesting that G_s protein activation of some cAMP-independent signaling pathway, although apparently not capable of activating $I_{Cl.PKA}$ in the absence of PKA phosphorylation, may play a role in amplifying the response of $I_{Cl.PKA}$ to PKA (334). Because of the absence of a direct G protein effect on $I_{Cl.PKA}$, and the fact that the amplitude of $I_{Cl.PKA}$ appears to reflect underlying adenylyl cyclase activity, $I_{Cl.PKA}$ represents a model system for studies of receptor-G protein-adenylyl cyclase-PKA pathways in heart. $I_{Cl.PKA}$ has been used to study the intracellular signaling pathways involved in the response to muscarinic (323, 324, 324, 432, 505, 507), α-adrenergic (179, 196, 321), β_2-adrenergic (177), histaminergic (190, 321), purinergic (344), and endothelin (199) receptor stimulation as well as the effects of thyroid hormone (156). Regulation of $I_{Cl.PKA}$ by PKC is discussed in section II*B3*.

C) BASAL ACTIVITY. Unlike other cAMP-dependent channels in heart, $I_{Cl.PKA}$ does not appear to be basally active in the absence of agonists, since protein kinase inhibitors generally do not appear to alter any Cl^--sensitive membrane conductance (190). Whether $I_{Cl.PKA}$ is basally active or not will be largely determined by the relative rates of basal adenylyl cyclase activity, basal PKA phosphorylation/dephosphorylation, as well as the level of endogenous phosphodiesterase activity in a cell. If basal PKA activity or adenylyl cyclase activity is significant, but phosphatase or phosphodiesterase activity dominates, then inhibition of endogenous phosphatases or phosphodiesterases alone should be sufficient to activate $I_{Cl.PKA}$. The initial test of this hypothesis used okadaic acid and microcystin to inhibit endogenous PP1 and PP2A in guinea pig myocytes, and these compounds failed to activate $I_{Cl.PKA}$ (190). It now seems clear that this type of experiment is strongly influenced by the experimental conditions and the extent to which intracellular dialysis may dilute any resting basal adenylyl cyclase or PKA activity in the cell. Subsequent studies have shown that okadaic acid or microcystin alone (175, 306) or phosphodiesterase inhibitors like IBMX alone (163) is capable of activating $I_{Cl.PKA}$, supporting the idea that the usual absence of basal $I_{Cl.PKA}$ activity may be attibutable to the predominance of basal phosphatase and/or phosphodiesterase activity in most cardiac cells. It would be interest-

ing to test the effects of phosphatase inhibitors on $I_{Cl.PKA}$ in nondialyzed cardiac myocytes using the perforated patch technique, since possible complicating effects of channel rundown may be prevented and the response to exogenously applied isoproterenol is significantly enhanced under these conditions (504).

Unlike CFTR channels, which are known to require PKA phosphorylation and ATP binding and hydrolysis to open, it remains unclear as to whether or not phosphorylation is required for L-type Ca^{2+} channels to open. Early studies suggested that basal current activation could be observed in the absence of agonists (169, 211), yet more recent single-channel studies suggest that phosphorylation may be required for channels to open (168, 322). In this regard, it is interesting to note that recent attempts to functionally express cloned L-type Ca^{2+} channel subunits have succeeded in producing currents that resemble in many aspects their native counterparts (for review, see Ref. 396). However, it has proven difficult to reconstitute PKA regulation of these channels, unless subunits are coexpressed along with the appropriate anchoring protein (140, 143). This might be taken as evidence that PKA phosphorylation per se is not required for basal Ca^{2+} channel activity, which is quite different from the situtation with CFTR channels, where PKA regulation of cloned channels is easily and consistently observed.

In functional studies, Hartzell and colleagues (121, 160) provided evidence that there may be significant basal phosphorylation of cardiac Ca^{2+} channels by endogenous kinases, but phosphorylation is probably not required for channels to open. Application of the protein phosphatase (PP1 and PP2A) inhibitors, okadaic acid and microcystin, to frog cardiomyocytes caused large increases in L-type Ca^{2+} current in the absence of β-adrenergic agonists, and such increases were dependent on intracellular ATP. However, in the absence of ATP, which prevented the effects of okadaic acid and microcytin, some basal Ca^{2+} current remained. Inhibition of PP2B (calcineurin) by inhibitory peptides or chelation of $[Ca^{2+}]_i$ did not mimic the effects of PP1 and PP2A inhibition. Interestingly, the increases in I_{Ca} induced by PP1 and PP2A inhibition were insensitive to concentrations of adenylyl cyclase or PKA inhibitors, which prevented isoproterenol stimulation of I_{Ca}, and insensitive to inhibitors of PKC, but were inhibited by nonspecific protein kinase inhibitors such as staurosporine and 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H-7). These results were interpreted to mean that an unknown protein kinase, termed PKX, is basally active in cardiac cells, and along with endogenous phosphatase activity sets the level of basal I_{Ca}. In a recent study, similar evidence supporting the role of PKX in basal regulation of I_{Ca} in mammalian cardiac myocytes has been obtained, and the possible role of PKX in regulating $I_{Cl.PKA}$ was also examined (175). Like the regulation of I_{Ca}, microcystin alone stimulated $I_{Cl.PKA}$, an effect which was ATP dependent, insensitive to inhibition of endogenous PKA or PKC, but was blocked by the nonspecific protein kinase inhibitors staurosporine or H-7. Although much remains to be learned about the identity of the mystery kinase PKX, these observations of basal protein kinase activity in cardiac myocytes may have relevance to some of the inconsistent effects that have been reported for some modulators of CFTR, such as genistein or phorbol esters (see sects. II*A2D* and II*B3*), whose effects may be dependent on PKA prephosphorylation of CFTR.

D) TYROSINE KINASE. The role of tyrosine kinases (TK) in the regulation of epithelial CFTR Cl^- channels is currently under investigation, and the mechanism of activation of CFTR by the TK inhibitor genistein remains unclear. Genistein activation of epithelial CFTR Cl^- channels was found not to depend solely on an elevation of cAMP, suggesting some direct involvement of TK in regulation of CFTR Cl^- channels (194, 376). However, other explanations for the effect of genistein on CFTR channels include indirect activation of CFTR by inhibition of protein phosphatases (347, 500) and a direct, TK-independent, interaction of genistein with the CFTR Cl^- channel protein, possibly at a NBD (126, 467, 474). Although both cAMP-dependent and -independent mechanisms of genistein action have been described, it seems clear that the ability of genistein to modulate CFTR channels by either mechanism requires PKA prephosphorylation of CFTR; genistein has little or no effect on PKA dephosphorylated CFTR channels (126, 347, 500).

Genistein has also been found to affect $I_{Cl.PKA}$ in native cardiac myocytes (51, 388, 436). In some of these studies, genistein alone failed to activate Cl^- currents but had a synergistic effect to potentiate Cl^- currents preactivated by isoproterenol, forskolin, or IBMX, whereas in other studies, genistein alone caused activation of a Cl^- conductance that resembled $I_{Cl.PKA}$. This variable ability of genistein to activate $I_{Cl.PKA}$ likely reflects important differences in the phosphorylation state of CFTR channels in dialyzed cells, since the level of basal endogenous PKA and phosphatase activity may vary markedly depending on the efficiency of internal dialysis.

The synergistic effects of genistein to potentiate PKA-preactivated $I_{Cl.PKA}$ in cardiac myocytes has recently been attributed to tyrosine dephosphorylation, which may somehow facilitate PKA-mediated phosphorylation of cAMP-dependent Cl^- channels, an action independent of genistein-induced elevation of cAMP or inhibition of serine/theonine phosphatases (389). However, the actions of genistein and orthovanadate were not extensively compared with other putative TK and protein tyrosine phosphatase (PTP) inhibitors in that study. Other evidence also raises more general doubts about the specificity of action of genistein and the potential role of TK in genistein-induced activation of CFTR Cl^- channels. Tyrosine phosphorylation was not detected in CFTR-trans-

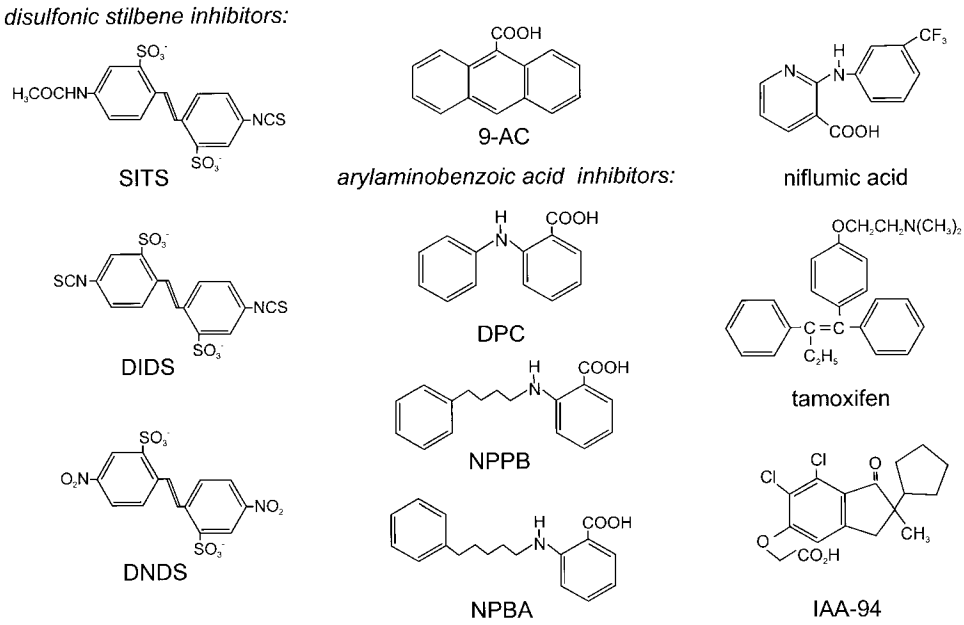


FIG. 3. Structures of commonly used anion transport inhibitors. SITS, 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid; DIDS, 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid; DNDS: 4,4′-dinitrostilbene-2,2′-disulfonic acid; 9-AC, anthracene-9-carboxylic acid; DPC, diphenylamine-2-carboxylic acid; NPPB, 5-nitro-2-(3-phenylpropylamino)benzoic acid; NPBA, 5-nitro-2-(4-phenylbutylamino)benzoic acid; IAA-94, indanyloxyacetic acid.

fected COS-7 cells (48). In cell-attached and excised patches from epithelial CFTR-transfected NIH/3T3 and Calu-3 cells, addition of cytosolic TK, $p60^{c\text{-}src}$, was shown to actually increase current amplitudes (116). In some studies, orthovanadate failed to antagonize genistein-induced CFTR currents, and other putative TK inhibitors like tyrphostin 47, herbstatin, or herbimycin A did not mimic the effects of genistein (474). Finally, French et al. (126) recently demonstrated that replacement of ATP with GTP, a poor substrate for TK, did not affect the ability of genistein to activate epithelial CFTR channels.

In another recent analysis of the synergistic effects of genistein on PKA-preactivated $I_{Cl.PKA}$, experiments were performed to distinguish between direct effects of genistein (and possibily TK) on $I_{Cl.PKA}$ from effects that might be due to TK modulation of some site in the cAMP-signaling pathway (178). Genistein was found to exert a synergistic action to not only potentiate $I_{Cl.PKA}$ activated by isoproterenol but also potentiated the activation of I_{Ca} and I_K by isoproterenol as well. Other nonspecific inhibitory effects of genistein and the weak TK inhibitor daidzein on I_{Ca} and I_K were noted as well. It would appear that the ability of genistein to activate $I_{Cl.PKA}$ or to potentiate the activating effects of other agonists in heart, like in epithelial cells, may be due to a direct, TK-independent interaction of genistein with CFTR at NBDB (126, 467), as well as by modulation of some unknown TK-sensitive site in the cAMP-signaling pathway (178). Evidence that TK directly regulates CFTR in heart remains equivocal.

3. Sensitivity to Cl^- channel blockers

The sensitivity of $I_{Cl.PKA}$ in heart to a various Cl^- channel antagonists is similar to epithelial CFTR channels (136, 373). Although some discrepancies have been reported, in general, $I_{Cl.PKA}$ is relatively insensitive to stilbene disulfonic acid derivatives like SITS, DIDS, and DNDS but is blocked by carboxylic acid derivatives like anthracene-9-carboxylic acid (9-AC) and diphenylamine-2-carboxylic acid (DPC), arylaminobenzoates like 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB), clofibric acid analogs, and sulfonylureas like glibenclamide (13, 161, 163, 386, 429, 439, 465, 499; see Fig. 3). Walsh and Wang (465) have carried out the most systematic comparison of Cl^- channel antagonists on $I_{Cl.PKA}$ in heart and tested their specificity by simultaneously examining their effects on PKA-stimulated L-type I_{Ca} as well. Although both 9-AC and DPC strongly inhibited $I_{Cl.PKA}$, these compounds also blocked PKA-stimulated I_{Ca}, suggesting important secondary nonspecific actions of these compounds. Some of the reported variable blocking effects of 9-AC on cardiac $I_{Cl.PKA}$ might also be due to an intracellular action of the compound to inhibit protein phosphatases (514). DIDS and indanyloxyacetic acid 94 (IAA-94) were poor inhibitors of $I_{Cl.PKA}$, but clofibric acid and its analogs, *p*-chlorophenoxy propionic acid and gemfibrozil, appeared to be the most specific inhibitors of $I_{Cl.PKA}$ in guinea pig myocytes.

In a recent study, the structural requirements necessary for arylaminobenzoate block of $I_{Cl.PKA}$ were examined (466). Increasing the length of the carbon chain between the benzoate and phenyl rings of the arylaminobenzoates resulted in a marked increase in potency, with IC_{50} values of 47, 17, and 4 mM for 2-benzylamino-5-nitrobenzoic acid, 5-nitro-2-(2-phenylethylamino)benzoic acid, and NPPB, respectively. Further increases in carbon chain length failed to affect potency. Block by external

NPPB was modulated by changes in extracellular pH, whereas block by internal NPPB was not. These results suggest that NPPB may be the most potent antagonist of $I_{Cl.PKA}$ yet examined. Further structure-function studies of Cl^- channnel antagonists on $I_{Cl.PKA}$ offer potential for the discovery of new potent antagonists that might exhibit a higher degree of selectivity among the different types of Cl^- channels present in cardiac muscle.

4. Species and tissue distribution

Electrophysiological studies indicate a significant species and tissue variability in the expression of $I_{Cl.PKA}$. In general, $I_{Cl.PKA}$ is most often found in adult ventricular, but not in atrial or sinoatrial nodal cells in guinea pig, rabbit, and cat (164, 427, 451, 513). In contrast, no evidence for $I_{Cl.PKA}$ has yet been found in adult canine (404), rat (98, 212), or mouse hearts (252); however see sect. II*E*), although $I_{Cl.PKA}$ has been reported in rat (436) and mouse (40) neonatal myocytes, suggesting that in some species $I_{Cl.PKA}$ may be developmentally regulated. Evidence for functional expression of $I_{Cl.PKA}$ in human heart is controversial (see sect. II*A5*B). Density of $I_{Cl.PKA}$ is higher in epicardial compared with endocardial cells in rabbit ventricle (427), and a recent study using in situ hybridization with CFTR specific probes combined with electrophysiological measurements of $I_{Cl.PKA}$ density has confirmed this pattern of expression in rabbit ventricle (444).

Because early studies generally failed to find $I_{Cl.PKA}$ in atrial myocytes, this has led to the notion that $I_{Cl.PKA}$ may have physiological relevance only in the ventricle. However, a small percentage of guinea pig atrial myocytes has been reported to express $I_{Cl.PKA}$ (282). In a timely study, James et al. (198) quantitated mRNA levels of CFTR in guinea pig atrium and ventricle and found strong correlations with $I_{Cl.PKA}$ densities, measured electrophysiologically. Specifically, mRNA levels and $I_{Cl.PKA}$ densities were lower (but not absent) in atrial cells and highest in ventricular epicardial cells compared with endocardial cells. This study set a new standard for quantitative mRNA studies in heart, and similar studies combining membrane current densities with quantitative RT-PCR of CFTR gene products in other species are needed to determine the generality of this pattern of tissue-specific myocardial expression of CFTR.

In earlier studies, RT-PCR using primers designed to amplify several different regions of CFTR was used to characterize CFTR expression in different species and areas of the heart (182, 251, 471). These results are illustrated in Figure 4. Of the three different regions of CFTR that were amplified, those corresponding to NBDA (E9-E13′, 550 bp) and M7-M12 (E14-E17′, 944 bp) were detected in ventricular tissue of rabbit and guinea pig heart and in atrium and ventricle of both human and simian hearts. Amplification of these products from dog atrium and ventricle and guinea pig and rabbit atium was not detected. These RT-PCR reactions were carried out in a single 30-cycle amplification, in contrast to James et al. (198) in which two amplifications generating extremely high sensitivity were performed. The lack of detectable CFTR expression in canine heart is consistent with the results of electrophysiological studies that have failed to observe $I_{Cl.PKA}$ in similar preparations (88, 404). Surprisingly, in virtually every cardiac tissue in which PCR was performed, regions corresponding to M1-M6 (E3-E7′) could be amplified to detectable levels. In all animal species, only a 681-bp product was detected, indicating exclusive expression of the exon 5− isoform, compared with control dog pancreas tissue in which the epithelial exon 5+ transcript (771 bp) is known to be expressed. Interestingly, in human atrium and ventricle and simian ventricle, both exon 5− and exon 5+ transcripts appear to be expressed. The detection of CFTR amplification products corresponding to M1-M6 segments of CFTR in tissues in which $I_{Cl.PKA}$ is not detected (e.g., canine) prompted speculation that since this region of CFTR is believed to contribute to the channel pore (see sect. II*A1*), such anomolous expression may be due to sequence homology of a conserved pore region in other types of Cl^- channels in heart (187). Although this remains a possible explanation, especially given the variety of different types of Cl^- channels that appear to be expressed in intracellular membranes of cardiac cells (see sect. VI), considerable future effort is needed to reconcile these apparently inconsistent expression patterns of CFTR thus far revealed by electrophysiological and molecular studies. It is possible that pseudogenes give rise to variant truncated transcripts for CFTR. Reverse transcription-polymerase chain reaction experiments designed to amplify CFTR specific segments that extend further than exon 7 were unsuccessful (Horowitz, unpublished observations). Future studies should include *1*) a more extensive examination of whether or not $I_{Cl.PKA}$ can be detected in canine myocardial tissue and in atrial tissue of several species, *2*) the use of quantitative RT-PCR to clearly establish relative CFTR mRNA levels, and *3*) the use of in situ hybridization and/or immunocytochemical techniques to clearly distinguish sarcolemmal CFTR expression from expression in internal membranes.

5. Recent controversies

A) Na^+DEPENDENCE In the original description of an isoproterenol-induced Na^+-dependent current, Na^+ was concluded to be a major charge carrier of the current since removal of extracellular Na^+ attenuated the response (99, 100). This Na^+ sensitivity was subsequently verified in other studies (163, 280), but rather than indicating substantial Na^+ permeability of the channels, it appeared to

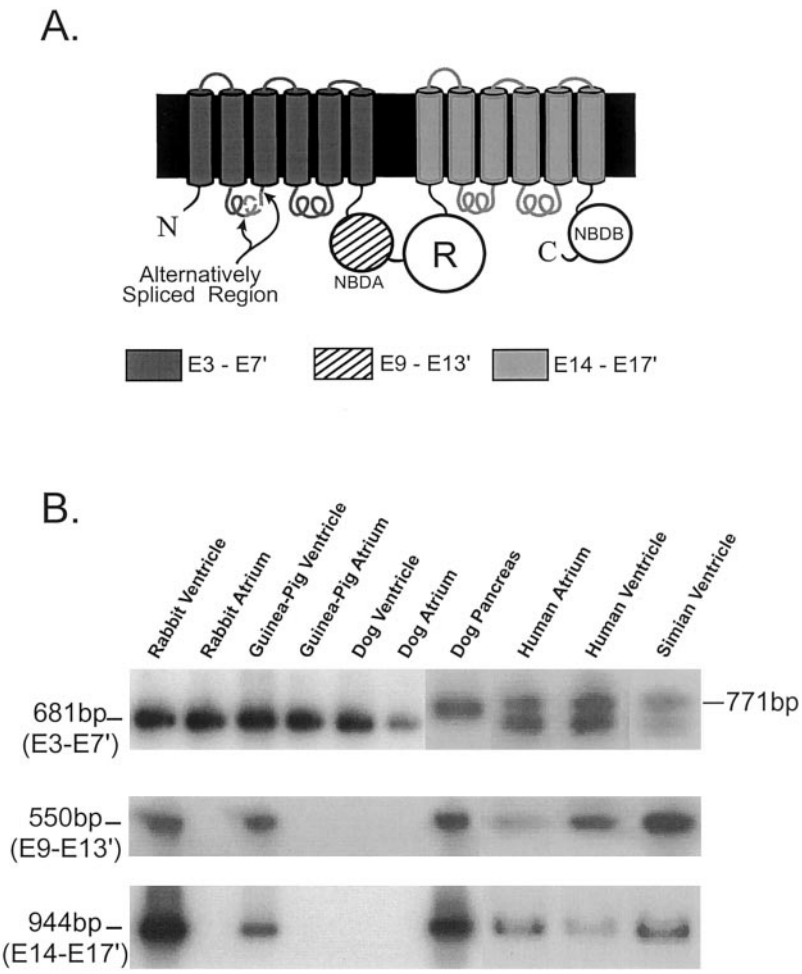


FIG. 4. RT-PCR amplification of heart-derived cDNA encoding NH_2-terminal (681 bp), nucleotide binding domain A (NBDA, 550 bp), and COOH-terminal (944 bp) segments of CFTR. *A*: predicted membrane topology of CFTR indicating the transmembrane segments, nucleotide binding domains (NBDA and NBDB), and regulatory domain (R). Oligonucleotide primers were designed to hybridize to sequences in exon 3 (sense) nucleotides (nt) 270–295 and exon 7 (antisense) nt 926–951 of CFTR cardiac (accession no. U40227) for the NH_2-terminal region (E3-E7′), exon 9 (sense) nt 1379–1399 and exon 13 (antisense) nt 1900–1929, for the NBDA region (E9-E13′), and exon 14 (sense) nt 2661–2681, exon 17 (antisense) nt 3585–3605 for COOH-terminal region (E14-E17′). *B*: representative agarose gel with amplification products from the RT-PCR reactions. Data demonstrate that rabbit, guinea pig, and canine hearts result in amplification of only the exon 5-deleted product (681 bp), whereas human and simian heart tissues yield both the exon 5-deleted and nondeleted (771-bp) products. RT-PCR amplification of mRNA from canine pancreas tissue only yielded the nondeleted product. Amplification products for other regions of CFTR are only detectable in rabbit and guinea pig ventricle, human atrium and ventricle, and simian ventricle. [Data compiled from Horowitz and co-workers (182, 187, 471).]

involve alteration of the I_{Cl} response at a regulatory site in the cAMP-dependent pathway. Attenuation of I_{Cl} by reduction of extracellular Na^+ was not accompanied by any significant change in the current reversal potential (163, 280), and a similar sensitivity to extracellular Na^+ was shown for β-adrenergic regulation of I_{Ca} (281). A later examination of the extracellular Na^+ sensitivity of $I_{Cl.PKA}$ suggested that it may be modulation by Na^+ at an intracellular site, possibly involving phosphorylation or dephosphorylation of Cl^- and Ca^{2+} channels (167). However, later key studies helped to eventually resolve the issue. Tareen et al. (433) suggested that most of the apparent extracellular Na^+ sensitivity occurs due to antagonism between Na^+ substitutes and isoproterenol at the level of the β-adrenoreceptor, since they could not observe Na^+ modulation using agents that activate the pathway beyond the β-receptor. Studies by Zakharov et al. (506) also showed that the observed extracellular Na^+ sensitivity may be related to muscarinic agonist activity of the Na^+ substitutes (Tris or tetramethylammonium) used earlier, thus leading to inhibition of adenylate cyclase activity via G_i protein activation. A recent study has confirmed that once these effects are prevented, changes in extracellular or intracellular Na^+ have no direct effect on $I_{Cl.PKA}$ (472).

B) FUNCTIONAL EXPRESSION IN HUMAN HEART. The molecular evidence presently available strongly suggests that CFTR message is expressed in both atrial and ventricular human myocardium (251, 471). In fact, RT-PCR products representing four distinct regions of CFTR all suggest expression of CFTR in both human as well as simian atrium and ventricle (Fig. 4). Moreover, in contrast to all other animal species yet examined, there is evidence for expression of both the exon 5+ as well as the exon 5− isoforms in human and simian myocardium. However, electrophysiological evidence for functional expression of CFTR Cl^- channels in human heart is weak. Only one study has provided evidence for the existence of $I_{Cl.PKA}$ in human myocytes (471), and that evidence was limited by the fact that only 27% of the atrial myocytes examined (average patient age 62 years) exhibited an intact adenylyl cyclase/PKA pathway (as assessed by measuring the response of I_{Ca} to forskolin). Of these, 63% responded to forskolin with the activation of a time-independent I_{Cl} that was DIDS insensitive. Consistent activation of $I_{Cl.PKA}$ by forskolin was observed in every simian ventricular myocyte

TABLE 1. *Properties of functionally identified sarcolemmal Cl^- channels in heart*

Current	Activation	Single Channel, pS	*I-V* ($[Cl^-]_o > [Cl^-]_i$)	*I-V* ($[Cl^-]_o = [Cl^-]_i$)	Permeability	Gene	Reference No.
$I_{Cl.PKA}$	AC-cAMP-PKA	7–13	Outwardly rectifying	Linear	$Br^- > Cl^- > I^-$	*CFTR*	13, 101, 102, 158, 163, 164, 191, 198, 251, 280, 304, 325
$I_{Cl.PKC}$	PKC	7–13	Outwardly rectifying	Linear	$Br^- > Cl^- > I^-$	*CFTR*	24, 63, 209, 290, 463, 497, 513
$I_{Cl.ATP}$	ATP_o (P_2 receptor)	~12	Outwardly rectifying	Linear	$Br^- > Cl^- > I^-$	*CFTR*?	96, 212, 252, 282
$I_{Cl.Ca}$	$[Ca^{2+}]_i$	~2	Outwardly rectifying	Linear	$I^- > Br^- > Cl^-$	*CLCA1*?	64, 139, 146, 217, 516–520
$I_{Cl.vol}$	Cell swelling	30–60	Outwardly rectifying	Outwardly rectifying	$I^- > Br^- > Cl^-$	*ClC-3*?	57, 91–93, 95, 97, 171, 219, 220, 400, 403, 445, 451
$I_{Cl.b}$	Basally active	30–60	Outwardly rectifying	Outwardly rectifying	$I^- > Br^- > Cl^-$	*ClC-3*?	91–95, 97, 445
$I_{Cl.ir}$	Basally active; cell swelling; pH_o	3–7	Inwardly rectifying	Inwardly rectifying	$Cl^- \geq Br^- > I^-$	*ClC-2*?	90, 130, 132, 147, 206, 316, 381, 413, 435

I-V, current-voltage; AC, adenylyl cyclase; PKA, protein kinase A; PKC, protein kinase C; CFTR, cystic fibrosis transmembrane conductance regulator; subscripts i and o, intracellular and extracellular, respectively.

examined. In 3 of 12 giant excised human atrial patches examined, unitary Cl^- channels activated by PKA catalytic subunit with a mean slope conductance of ~14 pS were observed. DIDS insensitivity, a 8- to 14-pS single-channel conductance, activation by PKA, and a linear current-voltage relationship in symmetrical Cl^- are all properties characteristic of cardiac and epithelial CFTR Cl^- channels (136, 353, 478), and inconsistent with the known properties of most other types of Cl^- channels in heart, including $I_{Cl.vol}$ (see Table 1).

However, a number of other studies have failed to detect $I_{Cl.PKA}$ in human atrial and ventricular cells, even under conditions in which I_{Ca} responses to stimulation of the adenylyl cyclase/cAMP/PKA pathway seemed intact (255, 327, 366, 371). Although these studies failed to detect $I_{Cl.PKA}$, exposure of cells to hypotonic solutions consistently revealed activation of $I_{Cl.vol}$. Failure to detect functional $I_{Cl.PKA}$ does not appear to be attributable to the usual vagaries that might inadvertently be associated with cell dialysis, since Li et al. (255) also failed to detect functional $I_{Cl.PKA}$ using the nystatin-perforated patch technique in human atrial cells. Forskolin activation of a Cl^--sensitive conductance was consistently observed in human atrial and ventricular myocytes, but only after cells were swollen after exposure to hypotonic solutions, and this was attributed to enhancement of $I_{Cl.vol}$ by forskolin, not to activation of $I_{Cl.PKA}$ (327). However, the adequacy of such a simple explanation seems uncertain at this time, since the only reported precedence of a stimulatory effect of cAMP on $I_{Cl.vol}$ describes variable biphasic stimulation/inhibition, monophasic stimulation, monophasic inhibition, or no response in canine atrial cells (88). In cultured chick myocytes, cAMP is reported to inhibit $I_{Cl.vol}$ (154); see sect. II*C4*). In another study in human atrial myocytes (371), isoproterenol alone failed to activate a Cl^--sensitive conductance and also failed to modulate the DIDS-sensitive $I_{Cl.vol}$ activated by hypotonic cell swelling. It is noteworthy that in studies in other species, macroscopic $I_{Cl.PKA}$ can be easily distinguished from $I_{Cl.vol}$ by its differential sensitivity to elevations of cAMP, pharmacological blockers, and kinetic and rectification properties (390, 451; see sect. II*C* and Table 1).

The only other study to examine unitary Cl^- channels in human myocardial cells utilized inside-out and outside-out membrane patches from human atrial myocytes (371), and these results seem to provide an additional level of confusion related to the question of functional expression of CFTR channels in human myocardium. Chloride-sensitive single-channel currents were activated by the application of positive pipette pressure to outside-out membrane patches, or the application of negative pipette pressure to inside-outside patches. Bath application of isoproterenol, forskolin, dibutyryl cAMP, or even PKA catalytic subunit (in the case of inside-out patches) failed to activate channels in the absence of applied pipette pressure and failed to affect channels that were preactivated by changes in pipette pressure. Surprisingly, the unitary conductance reported for these channels (~9 pS) and their linear current-voltage properties in symmetrical Cl^- more closely resemble the properties of channels known to be associated with CFTR (136) than channels usually associated with $I_{Cl.vol}$ (see sect. II*C* and Table 1), although the channels were reported to be inhibited by DIDS. A possible complicating factor in these studies is the possibility that cAMP activation of CFTR channels may be influenced by the actin cytoskeleton. Cytochalasin D alone reportedly activates whole cell CFTR currents, addition of actin alone to excised inside-out patches activates unitary CFTR channels, and long-term exposure to cytochalasin D which can derange the actin cytoskeleton prevents the cAMP-dependent activation of CFTR (40).

Other complicating factors in studies of human myocardial tissue are alterations as a result of disease, drugs, or age of patients and practical difficulties usually associated with obtaining viable human myocardial samples in a timely fashion for enzymatic dispersion. Human atrial

myocytes isolated from pediatric patients (aged 1 day to 11 yr) also failed to exhibit detectable $I_{Cl.PKA}$, even though many cells appeared to express a basally active Cl^- conductance that was inhibited by 9-AC (25). It seems clear from animal studies that CFTR expression is highest and $I_{Cl.PKA}$ is most consistently detected in ventricular myocytes, compared with atrial myocytes, where only 10–15% of the cells may express CFTR (cf. Ref. 198). This factor could certainly explain some of the inconsistent results that have been reported for functional expression of $I_{Cl.PKA}$ in human heart. Most studies to date have been performed on human atrial myocytes. The exception is a study by Oz and Sorota (327), which also failed to detect $I_{Cl.PKA}$ in human ventricular myocytes, although these myocytes were isolated from failing human hearts. A very recent study in human ventricle suggests that action potential shortening in response to stimulation of β_3-adrenoceptors may be mediated by activation of CFTR Cl^- channels, since such action potential changes were not observed in ventricular biopsies obtained from ΔF508/ΔF508 cystic fibrosis patients undergoing cardiopulmonary transplantation (243).

Because virtually all of the existing molecular data supporting expression of CFTR in human myocardium have come from only one laboratory, additional independent studies are needed, which include quantitative measurements of mRNA levels and immunocytochemical studies of protein expression patterns, to corroborate the existing molecular evidence. Future functional studies should seek to minimize the possible confounding effects of disease, drugs, or age of patients; utilize experimental conditions that provide more accurate identification of macoscopic Cl^- currents combined with careful measurements of single-channel properties; and focus more on human ventricular myocytes, which may exhibit higher density and more consistent expression of the CFTR gene product than atrial myocytes.

C) FUNCTIONAL SIGNIFICANCE OF EXON 5. Existing molecular evidence suggesting exclusive expression of the exon 5− isoform of CFTR in the heart of most animal species examined to date raises the obvious question of functional significance. Four cytoplasmic loops (CL) (ignoring the large NBDA and R-domain region) connect the transmembrane domains of CFTR (Fig. 4), which are expected to be ~55–65 amino acids in length and generally are highly conserved between different species (79, 354). It has been suggested that due to their highly lipophilic nature, the CL may interact with other regions of CFTR or other proteins (430), but the functional significance of the CL is only beginning to be understood. Exon 5 encodes 30 amino acids in first cytoplasmic loop (CL1), but their functional role is unknown. On the basis of mutagenesis experiments, CL2 and CL3 have been proposed to help stabilize the full conductance state of CFTR (378, 492), whereas CL4 appears to affect the responsiveness to regulatory stimuli (377). It has been reported that an engineered epithelial exon 5− isoform of CFTR fails to generate functional channels when expressed in HeLa cells, presumably due to defective intracellular processing, suggesting that exon 5− transcripts may generate nonfunctional proteins (77). In addition, exon 5− isoforms were found to be the most abundant alternatively spliced transcripts in mice. A subsequent study confirmed that the engineered epithelial exon 5− isoform exhibited a processing defect, becoming trapped in intracellular membranes in HEK 293 cells, but retained some functional Cl^- channel activity when isolated and incorporated into lipid bilayer membranes (493). These exon 5− CFTR channels exhibited an average P_o significantly smaller ($P_o < 0.01$) than wild-type channels (P_o ~0.3), and channels exhibited a small subconductance state (2–3 pS) more frequently compared with wild-type channels. These results suggest that CL1 may be involved in both intracellular processing as well as the conductance properties of the channel.

The relevance of these results obtained using an engineered exon 5− isoform of the epithelial CFTR channel to cardiac expression of an exon 5− isoform of CFTR is presently unknown. An engineered exon 5− epithelial isoform may not be exactly equivalent to the cardiac exon 5− spliced isoform, since in addition to absence of exon 5, there are also additional differences of ~10% in amino acid identity (158). As previously discussed (136), functional studies of unitary CFTR channels in native cardiac myocytes reveal strong similarities in conductance and gating properties, ATP hydrolyis, and regulation by phosphorylation compared with epithelial CFTR channels, although exon 5− might account for the apparent lower density of expression observed in cardiac cells. In fact, cDNA encoding the rabbit cardiac exon 5− isoform or the epithelial exon 5+ isoform are both robustly expressed in *Xenopus* oocytes, resulting in the appearance of $I_{Cl.PKA}$ with similar membrane current densities and properties (158, 497). Figure 5 illustrates single-channel properties associated with expression of the rabbit cardiac exon 5− isoform in *Xenopus* oocytes. In inside-out membrane patches, channels were activated by exposure to PKA catalytic subunit and MgATP (Fig. 5*A*). Once phosphorylated, channel activity depended only on the presence of MgATP, suggesting low endogenous phosphatase activity in the detached membrane patches. The voltage dependence of channels preactivated by PKA catalytic subunit and MgATP is shown in Figure 5*B*, and the current-voltage relationship is plotted in Figure 5*C*. In this example, the channels had a slope conductance of 7.2 pS, were linear, and reversed near 0 mV, the predicted value of Cl^- equilibrium potential (E_{Cl}) in symmetric Cl^-. In cell-attached membrane patches (Fig. 5, *D–F*), similar channels were activated by exposure of oocytes to forskolin (9.5 ± 0.8 pS, $n = 5$) or to the phorbol ester phorbol 12,13-dibuytrate (PDBu) (10.6 ± 0.4 pS, $n = 5$). Although

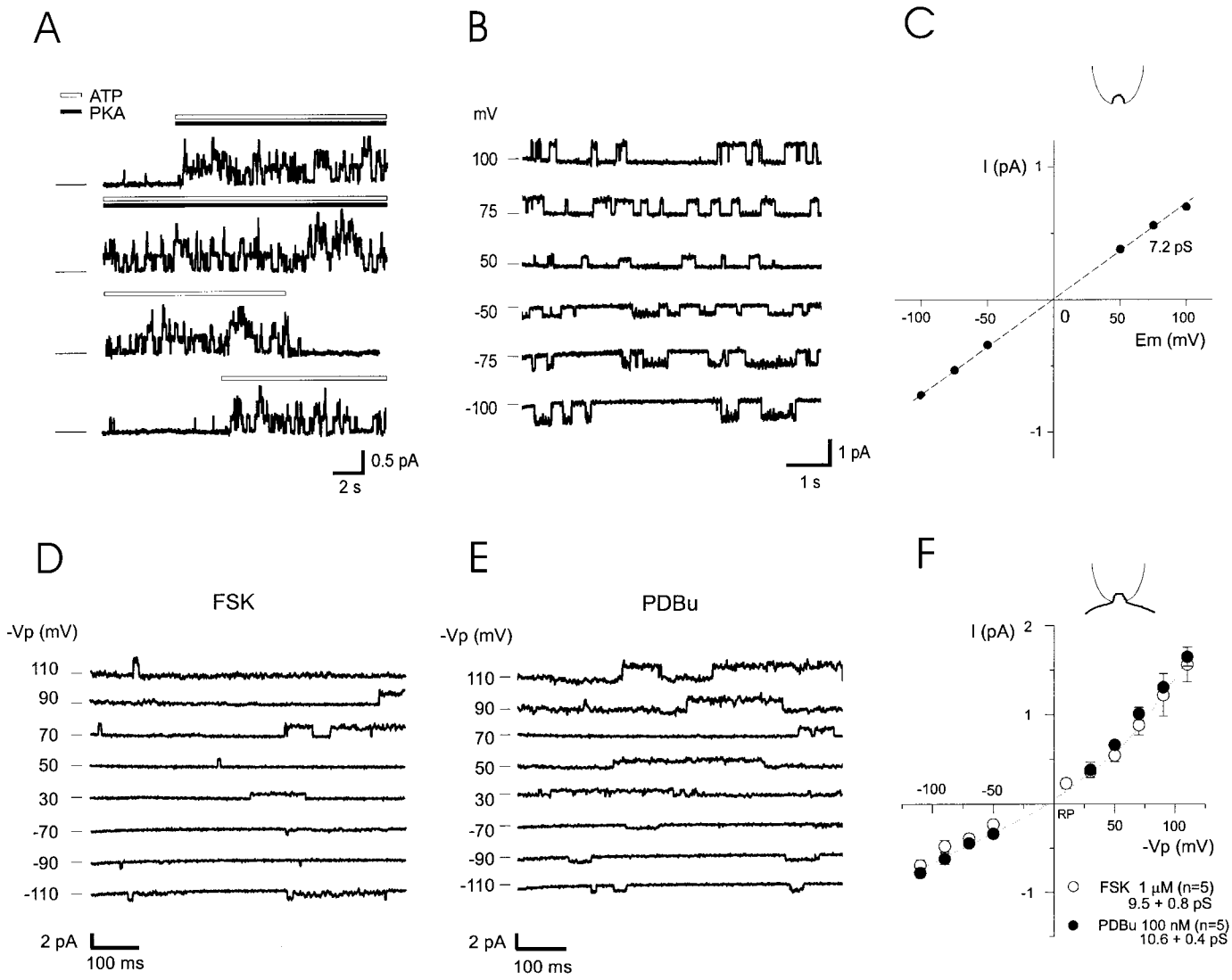


FIG. 5. Recombinant rabbit cardiac exon 5− CFTR channels in inside-out (*A*—*C*) and cell-attached (*D* and *E*) membane patches from *Xenopus* oocytes. In inside-out patches, channels were initially activated by 100 nM PKA catalytic subunit (100 nM) and 0.5 mM MgATP (*A*). Channels closed upon washout of PKA and MgATP but could be reopened by exposure to MgATP alone. Representative channel openings at different patch potentials (V_p) in the presence of PKA and MgATP are shown in *B*, and single-channel conductance in this patch was 7.2 pS (*C*). In cell-attached membrane patches, CFTR channels were opened by bath application of 1 mM forskolin (FSK; *D*) or 100 nM phorbol 12,13-dibutyrate (PDBu) (*E*). Mean single-channel conductance for forskolin-activated channels was 9.5 ± 0.8 pS (n = 5) and for PDBu-activated channels was 10.6 ± 0.4 pS (n = 5) (*F*). For cell-attached patches, pipette solution contained (in mM) 100 *N*-methylglucamine chloride (NMG-Cl), 5 CsCl, 2.5 $MgCl_2$, and 10 HEPES, pH 7.3; bath solution contained modified ND-96 solution (in mM: 96 NaCl, 2.0 KCl, 1.8 $CaCl_2$, 1.0 $MgCl_2$, 100 niflumic acid, and 5.0 HEPES, pH 7.4). For inside-out patches, pipette solution (external solution) had same composition as bath solution for cell-attached mode; bath (internal) solution contained (in mM) 100 NMG-Cl, 6 CsCl, 2 $MgCl_2$, 5 EGTA, and 10 HEPES, pH 7.3.

an extensive analysis of channel properties associated with expression of recombinant cardiac exon 5− CFTR in oocytes has not yet been performed, the conductance, gating, and regulation of these channels appear to closely resemble those of unitary CFTR channels described in native cardiac myocytes (101, 102) and epithelial exon 5+ CFTR channels expressed in stable cell lines (150), in contrast to the reported properties of reconstituted epithelial exon 5− engineered CFTR channels recorded in bilayers (493).

It is possible that alternative splicing may be involved in cell-specific targeting of CFTR (31). The cardiac exon 5− isoform thus may be properly processed and functionally expressed in cardiac myocytes, in contrast to many other types of mammalian cells, where the protein may be improperly processed. In addition, the protein-trafficking

system in nonpolarized cardiac cells may be different from either native epithelial cells or stable cell lines. The fact that exon 5 contains two putative PKC phosphorylation sites also suggests the possibility that there may exist characteristic differences in PKC regulation of the cardiac (exon 5−) and epithelial (exon 5+) isoforms. However, recent measurements of macroscopic currents associated with expression of the cardiac and epithelial isoforms expressed in oocytes suggest no overt differences in their response to stimulation of PKC (497; see sect. II*B3*).

6. Physiological and pathophysiological role

The predicted effects of $I_{Cl.PKA}$ activation to shorten action potential duration and under some experimental conditions to induce or modulate automaticity have been verfied experimentally. These effects and their physiological and pathophysiological relevance are discussed in section V. However, eventual understanding of the actual functional and clinical significance of this class of cardiac anion channels depends to a great extent on resolution of the existing ambiguities relating to expression of CFTR channels in human heart. It is not clear whether or not defects in cardiac CFTR function or expression have any clinical significance in cystic fibrosis (CF) patients. Comparative functional and molecular studies of $I_{Cl.PKA}$ and CFTR transcripts in myocytes from normal and CF patients have yet to be performed. A recent report (243) suggests that stimulation of β_3-adrenoceptors reduces action potential duration in human myocytes from normal patients, but not in myocytes obtained from ΔF508/ΔF508 CF patients, but further studies are needed to confirm that this difference is due to altered expression or function of cardiac CFTR channels. It is not known whether the myriad of CF-associated mutations that cause defects in CFTR production, processing, regulation, or conductance properties in epithelial cells (477) necessarily result in the same types of defects in cardiac myocytes, which may process proteins very differently than polarized epithelial cells. No efforts have yet been made to determine whether or not significant electrocardiogram abnormalities, especially during strong sympathetic stress, may occur in CF patients. Early hopes of exploiting the mouse CFTR-knock-out model to address these questions were reduced by the failure to detect functional $I_{Cl.PKA}$ in mouse ventricular myocytes (252). However, new revelations suggesting that both $I_{Cl.ATP}$ and $I_{Cl.PKC}$ may be mediated by CFTR channels (see sect. II*E*), combined with new molecular data confirming expression of CFTR in mouse heart (96), suggest that this model may in fact be useful to exploit in future functional studies. Furthermore, future studies assessing functional expression of CFTR Cl^- channels in human myocardial cells need to consider the possible role of PKC, as well as PKA phosphorylation, in the regulation of channel activity (209).

B. Cl^- Channels Activated by PKC

1. Macroscopic currents

The first evidence that activation of PKC in heart might be linked to activation of I_{Cl} was obtained in guinea pig ventricular myocytes by Walsh (463). Exposure of cells to phorbol 12-myristate 13-acetate (PMA) or PDBu, to stimulate PKC, activated a time-independent Cl^--sensitive current. The current-voltage relation for the PKC-activated current was linear in symmetric Cl^- and was reversibly inhibited by the skeletal muscle Cl^- channel blocker the *S*-(−)-enantiomer of 8-chlorophenoxypropionic acid (65). Dialysis of cells with partially purified PKC resulted in the activation of a similar but larger current after addition of PDBu, and in the presence of a β-adrenergic receptor antagonist, norepinephrine activated a similar current, suggesting that $I_{Cl.PKC}$ might be linked to α-adrenergic receptor stimulation. Subsequent studies further characterized the macroscopic properties of $I_{Cl.PKC}$ in guinea pig (387, 464) and feline ventricular myocytes (513). These studies were in general agreement, indicating that $I_{Cl.PKC}$ is selective for Cl^-, exhibits a linear current-voltage relation in symmetrical Cl^-, and is inhibited by 9-AC but not by DIDS. Because these properties closely resemble those of $I_{Cl.PKA}$, the possibility that stimulation of PKA and PKC might activate the same population of Cl^- channels was considered. Small apparent differences in anion selectivity of $I_{Cl.PKC}$ ($SCN^- > I^- > Br^- \sim Cl^-$) compared with $I_{Cl.PKA}$ in which I^- was generally thought to be equally or slightly less permeable than Cl^- (325) was considered evidence that PKA and PKC might activate different Cl^- channels (464). However, the observed changes in reversal potential observed in these experiments were small, and it is now known that determining relative permeabilities to Cl^- and I^- for $I_{Cl.PKA}$ are more complicated and are dependent on the direction of anion transport (424) and the fact I^- can readily enter open channels but leaves them slowly (84).

Zhang et al. (513) suggested that stimulation of PKC or PKA activated the same population of Cl^- channels in feline ventricular myocytes. This was based on similarities in macroscopic currents and the fact that PKC- and PKA-activated currents were additive when submaximal concentrations of agonists were used, but maximal activation by one agonist occluded activation by the other agonist. A similar conclusion was reached in further comparative studies of $I_{Cl.PKA}$ and $I_{Cl.PKC}$ in guinea pig ventricular myocytes (387).

In two different preparations, ANG II has been reported to activate a macroscopic I_{Cl}, which might be mediated by activation of PKC. In rabbit sinoatrial nodal cells, ANG II activated a 9-AC- and DPC-sensitive I_{Cl} that could be prevented by the AT_1 receptor antagonist losartan and by a PKC inhibitor (26). In rabbit ventricular

myocytes, ANG II-activated I_{Cl} was inhibited by the ANG II receptor antagonist saralasin and by the stilbene derivative DNDS (300). Angiotensin II-activated I_{Cl} also seemed to be dependent on $[Ca^{2+}]_i$ in this study, since strong intracellular Ca^{2+} buffering prevented activation, suggesting the possible involvement of a Ca^{2+}-dependent PKC isozyme. Unfortunately, however, neither of these studies provided sufficient characterization of the properties of the ANG II-induced I_{Cl} to definitively conclude that these may be the same channels activated by phorbol esters, and whether or not they may be similar to or distinct from $I_{Cl.PKA}$.

2. Unitary currents

Additional direct evidence indicating that both PKC and PKA activate the same population of Cl^- channels in heart came from an investigation of unitary currents activated by PKA and PKC in cell-attached patches from guinea pig ventricular myocytes (63). Single Cl^- channels elicited by phorbol esters in cell-attached patches exhibited pharmacological and kinetic properties nearly identical to those previously reported for PKA-activated Cl^- channels in heart. In particular, the unitary Cl^- channels had a mean conductance close to 9 pS, were not blocked by DIDS, had P_o values similar to PKA-activated channels, were outwardly rectifying in asymmetric Cl^- but linear in symmetric Cl^-, and were voltage independent. Finally, in patches containing Cl^- channels preactivated with a phorbol ester, subsequent bath application of the phosphodiesterase inhibitor IBMX resulted in two- to sixfold increases in P_o without any evidence for the activation of a separate population of channels with a different unitary conductance.

3. CFTR regulation by PKC

Cystic fibrosis transmembrane conductance regulator channels contain several consensus phosphorylation sites for PKC, and epithelial channels in inside-out membrane patches have been shown to be activated by PKC (24, 423). The CFTR is phosphorylated in vitro by PKC (24), and serine-686 and serine-790 in the R domain seem to be preferential sites for PKC phosphorylation, although other phosphorylation sites may also be phosphorylated (335). However, the functional effects of mutations of these residues on PKC regulation of epithelial CFTR channels have not been assessed, although mutation of individual PKC sites has been reported to have little effect on activation by PKA (483).

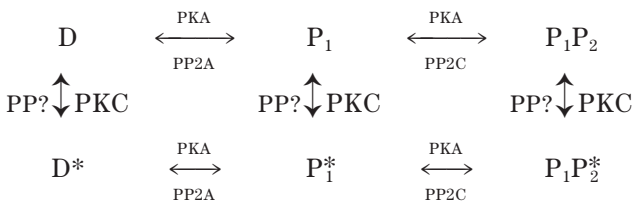
The ability of purified PKC isozymes or phorbol esters alone to activate epithelial CFTR Cl^- channels seems to be variable (24, 423, 485), and a more consistent finding is that PKC stimulation potentiates the rate and magnitude of subsequent PKA stimulation (419, 423, 485, 502). A similar variability in the ability of phorbol esters to activate cardiac CFTR channels has also been observed. For example, the addition of phorbol esters alone to guinea pig ventricular myocytes has been reported to activate robust Cl^- currents, with characteristic properties of CFTR (387), yet in other studies in the same preparation, phorbol esters alone failed to activate any significant Cl^- current (321).

The ability of PKC stimulation to potentiate PKA activation of CFTR may be due to PKC facilitatation of PKA phosphorylation (74, 335). Thus some of the variable responses reported for phorbol ester activation of CFTR might be explained by differences in experimental conditions, the extent to which intracellular dialysis may dilute resting basal adenylyl cyclase, PKA or phosphatase activity in intact cells (see sect. II*A2*C), and the extent to which these proteins remain active and membrane associated in detached membrane patches. It has been suggested, in the case of epithelial CFTR channels, that PKC phosphorylation may even play an obligatory role for acute activation of CFTR by PKA stimulation (209). In excised membrane patches from CHO cells, CFTR responses to PKA were observed to progressively rundown and become refractory with time. The addition of PKC and DiC_8 alone, although unable to directly activate channels, restored the responsiveness of channels to PKA. This apparent dependence of acute PKA activation of CFTR on PKC prephosphorylation was consistent with earlier results that showed that prolonged (4 h) incubation of T84 cells with phorbol esters that caused significant downregulation of endogenous PKC activity, eliminated subsequent activation of CFTR channels by 8-bromo-cAMP (74). Two interesting conclusions arise from these studies: *1*) the degree of basal PKA prephosphorylation of CFTR may account for the reported variability in acute responses to PKC stimulation, and *2*) the degree of basal PKC prephosphorylation of CFTR may account for reported variability in acute responses of CFTR to PKA stimulation. It is clear that synergistic interactions between PKA and PKC phosphorylation play an important role in the regulation of epithelial CFTR channels, but the detailed nature of these interactions has yet to be elucidated.

Interactions beween PKA and PKC in the regulation of cardiac exon 5− CFTR channels have recently been studied in both native cardiac myocytes (290) and in *Xenopus* oocytes expressing the recombinant cardiac (exon 5−) transcript (497). In guinea pig myocytes, stimulation of endogenous PKC with PDBu alone failed to activate CFTR Cl^- currents, even when intracellular dialysis was limited with the perforated patch-clamp technique. Phorbol esters did, however, activate CFTR currents in cells preexposed to low concentrations of isoproterenol and increased the magnitude of the response to supramaximal concentrations of isoproterenol; effects which were blocked by the purportedly selective PKC inhibitors, chelerythrine or bisindolylmaleimide. In

addition, preexposure of cells to chelerythrine or bisindolylmaleimide significantly reduced the magnitude of CFTR Cl^- currents subsequently activated by isoproterenol. These data confirm the synergistic effects of these kinases on CFTR in cardiac myocytes and suggest that acute reponses to both PKC and PKA seem to be highly dependent on the existence of basal phosphorylation by the opposite kinase.

In *Xenopus* oocytes expressing recombinant cardiac (exon 5−) CFTR channels, exposure to phorbol esters alone activated robust Cl^- currents in CFTR-injected oocytes that were absent in control water-injected oocytes (497). The ability of phorbol esters to activate CFTR Cl^- channels was inhibited by the specific PKC inhibitor bisindolylmaleimide and was dependent on endogenous PKA activity, since it was prevented by pretreating oocytes with a specific PKA inhibitor adenosine 3′,5′-cyclic monophosphothioate (Rp-cAMPS). Block of endogeneous PKA activity in combination with several protein phosphatase inhibitors was used to trap PKA-activated CFTR channels into different functional phosphorylation states to test the hypothesis that PKC stimulation of CFTR may be due to facilitation of PKA phosphorylation. In this study (497), intraoocyte injection of 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA), or pretreatment of oocytes with BAPTA-AM, nearly completely prevented dephosphorylation of CFTR currents activated by cAMP, an effect consistent with inhibition of PP2C by chelation of the required intracellular cofactor Mg^{2+}. With the use of the sequential PKA phosphorylation model previously proposed by Hwang et al. (190) for cardiac CFTR channels (Fig. 2), the effects of PKC stimulation were tested on channels trapped into either the dephosphorylated (D) state, the partially (P_1) phosphorylated state, or the fully (P_1P_2) phosphorylated channel state



In this model, channels trapped in the D state or in either of the PKA phosphorylated states can be additionally phosphorylated by PKC, as designated by asterisks. The role of endogenous PP in dephosphorylation of PKC sites is unknown (PP?). After inhibition of endogenous PKA, PKC stimulation alone failed to activate channels, confirming that channel openings require PKA phosphorylation of distinct sites on the R domain, while ATP hydrolysis of the two nucleotide binding domains is directly coupled to channel gating (136, 137). When channels were trapped into the P_1 state, after inhibition of PKA and PP1 and PP2A, or trapped into the P_1P_2 state, after inhibition of PP2C, stimulation of CFTR channels by PKC was prevented. These results suggested that the stimulatory effects of PKC on CFTR channels only occur when D* → P_1* or P1* → P_1P_2* transitions were allowed, functionally confirming earlier suggestions that PKC phosphorylation of CFTR Cl^- channels may facilitate PKA-mediated phosphorylation (74, 335), possibly by causing a conformational change in the R domain. The importance of PKC phosphorylation of serine-686 and serine-790 was also confirmed using site-directed mutagenesis. S686A and S790A mutant CFTR channels exhibited a significantly smaller stimulation by phorbol esters, but other additional sites may also be important because these mutations did not completely eliminate the response to phorbol esters.

In summary, the preponderance of evidence suggests that the $I_{Cl.PKC}$ observed in cardiac cells from a variety of species can be attributed to PKC regulation of CFTR channels (see Table 1). To date, there is very little evidence suggesting that PKC stimulation in heart leads to activation of a unique class of anion channels distinct from CFTR. Because α_1-adrenergic receptors are well known to activate phospholipase C leading to formation of inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG) and subsequent activation of PKC in many types of cells (149) including heart (113, 434), it is expected that PKC regulation of CFTR in heart might be mediated by an α-adrenergic signaling pathway. However, recent studies of the effects of α-adrenergic receptor stimulation on cardiac CFTR channels suggest that this is probably not the case. α-Adrenergic receptor stimulation appears to inhibit activation of $I_{Cl.PKA}$ in guinea pig ventricular myocytes by a pathway that involves inhibition of β-adrenergic signaling at a site upstream of G protein-dependent activation of adenylate cyclase, an effect independent of activation of PKC (179, 196, 321). Because α-adrenergic receptor stimulation in these studies also failed to modulate $I_{Cl.PKA}$ activated downstream of this site by forskolin or PKA catalytic subunit, it seems unlikely that PKC modulation of CFTR channels in heart is regulated through an α-adrenergic signaling pathway (cf. Fig. 1). Protein kinase C regulation of cardiac CFTR may instead involve a purinergic signaling pathway (see sect. II*E*) and/or other pathways possibly including angiotensin receptor activation (26, 300).

C. Cl^- Channels Regulated by Cell Volume

Volume-regulated anion channels (VRAC), as described in T lymphocytes (36), are now known to be ubiquitously expressed in mammalian cells and play an important role in cell volume homeostasis. The activation of $I_{Cl.vol}$ is believed to provide one of the initial triggers

linking cell swelling to the subsequent loss of osmolytes and water resulting in a regulatory volume decrease (RVD) (141). In most cells, an increase in cell volume activates outwardly rectifying anion channels (VSOAC), which inactivate at positive membrane potentials, and exhibit an anion selectivity of $SCN^- > I^- > NO_3^- > Br^- \geq Cl^- >$ gluconate (for reviews, see Refs. 313, 317, 412). Although exposure to hypotonic solutions is the most common technique used to swell cells and activate $I_{Cl.vol}$, in some cells a similar outwardly rectifying anion current appears to develop spontaneously under isotonic conditions, which can be suppressed by exposure to hypertonic solutions. Although these hypotonically or spontaneously activated Cl^- currents are often referred to as $I_{Cl.swell}$, the term $I_{Cl.vol}$ seems more appropriate because they are also regulated by cell shrinkage. Despite considerable molecular efforts in recent years, the identification of the protein responsible for $I_{Cl.vol}$ has remained elusive and has even become the subject of considerable recent controversy (52, 411).

1. Macroscopic currents and RVD

The first evidence for expression of $I_{Cl.vol}$ in heart was made in canine atrial (400) and ventricular (445) myocytes and rabbit atrial and sinoatrial myocytes (153). In canine atrial myocytes, spontaneously activating outwardly rectifying Cl^- currents were observed after patch rupture, whereas hypotonic solutions were used to swell cells and activate $I_{Cl.vol}$ in canine ventricular myocytes. In rabbit myocytes, the application of positive patch pipette pressure was used to inflate cells and activate $I_{Cl.vol}$. The macroscopic currents activated by cell swelling in these studies exhibited similar properties, including sensitivity of reversal potential to changes in the Cl^- gradient, pronounced outward rectification, and sensitivity to block by SITS, DIDS, NPPB, and high concentrations of 9-AC. Despite the apparent similarities in macroscopic currents, it remains to be determined whether or not the different types of stimuli necessarily activate the same type of anion channels in these studies. In rabbit atrial and sinoatrial cells, $I_{Cl.vol}$ was further characterized by an anion permeability sequence of $SCN^- > I^- > NO_3^- > Br^- > Cl^- > F^-$, and in both rabbit atrial and sinoatrial cells and canine ventricular myocytes, the activation of macroscopic $I_{Cl.vol}$ was reported to be insensitive to $[Ca^{2+}]_i$ and inhibitors of PKA, thus clearly establishing this membrane current to be independent of $I_{Cl.PKA}$ and $I_{Cl.Ca}$. In contrast, in canine atrial cells, the spontaneously activated Cl^- current, which was found to be correlated with changes in cell volume, could be augmented by isoproterenol (400). Subsequent studies in dog atrial myocytes found a similar outwardly rectifying Cl^- sensitive current that could be activated by hypotonic cell swelling (401) or cell inflation (87).

Exposure of cultured chick heart cell aggregates to hypotonic solutions has been shown to cause an initial cell swelling and then a subsequent RVD in the continued presence of hypotonic solutions (345). The RVD appears to be mediated by loss of amino acids but also is Cl^- dependent. $I_{Cl.vol}$ activated by hypotonic cell swelling has been characterized in cultured chick heart cell aggregates and shown to partially mediate RVD (512). Changes in cell volume and whole cell currents recorded simultaneously revealed that $I_{Cl.vol}$ activated by hypotonic solutions, elevation of intracellular osmolarity, isosmotic urea uptake, or cell inflation were indistinguishable in terms of time course, reversal potential, whole cell conductance, and response to a number of channel blockers (510). Further experiments suggested that cytoskeletal changes in F-actin may be involved in the volume transduction processes associated with activation of $I_{Cl.vol}$ (509). In canine ventricular myocytes, the activation of macroscopic $I_{Cl.vol}$ was shown to be altered by cationic and anionic amphipaths, suggesting that changes in membrane tension may be involved in the volume transduction process (445).

$I_{Cl.vol}$ has also been described in guinea pig atrial and ventricular myocytes. Osmotic swelling of guinea pig cardiac myocytes causes activation of an outwardly rectifying, anion-selective current with a conductance and permeability sequence of $I^- \sim NO_3^- > Br^- > Cl^- > Asp^-$ (451). This current was clearly distinguishable from $I_{Cl.PKA}$ by its marked outward rectification in symmetric Cl^- and inhibition by tamoxifen and DIDS. $I_{Cl.vol}$ could be elicited in >90% atrial myocytes studied but in only 34% ventricular myocytes, whereas $I_{Cl.PKA}$ could be elicited in <10% atrial myocytes and >90% ventricular myocytes. Another method for distinguishing macroscopic $I_{Cl.vol}$ from $I_{Cl.PKA}$ in the same cells is the characteristic time-dependent relaxation or inactivation of $I_{Cl.vol}$ observed at positive membrane potentials, which is not observed with $I_{Cl.PKA}$. Such inactivation is characteristic of $I_{Cl.vol}$ described in a variety of epithelial cells (286, 397, 487). A similar inactivation of $I_{Cl.vol}$ at positive membrane potentials is observed in guinea pig ventricular myocytes (390), which clearly distinguishes these currents from those mediated by $I_{Cl.PKA}$.

Although $I_{Cl.vol}$ is activated by cell swelling and may play a role in RVD, a number of other channels and transporters (see sect. IV) in heart also seem to be modulated by changes in cell volume. These include delayed rectifier K^+ channels (348, 370), K_{ATP} channels (454), Na^+-K^+ pump (370), and Na^+/Ca^{2+} exchange (489; for review, see Ref. 450). In addition, stretch-activated ion channels (SAC), which are believed to be directly gated by mechanical stimulation, primarily cation selective, and sensitive to block by gadolinium (Gd^{3+}), may also contribute to cell volume regulation in heart (184). It is not yet exactly clear whether or not volume-activated channels should be considered stretch activated, since they may not necessarily be responsive to the same stimuli, and the underlying mechanisms of activation may be dif-

ferent. Recent studies using high-resolution digital video microscopy of isolated rabbit ventricular myocytes have provided new information on the relative role of SAC and $I_{Cl.vol}$ in cell volume regulation (418). Gd^{3+}, a blocker of cation SAC, reduced the degree of cell swelling induced by hypotonic solutions, whereas 9-AC, a blocker of $I_{Cl.vol}$, significantly increased the degree of cell swelling induced by hypotonic solutions, suggesting that activation of SAC and $I_{Cl.vol}$ during cell swelling have opposite effects on cell volume regulation. Moreover, this study provided evidence that possible stretch-activated increases in Ca^{2+} influx or modulation of K_{ATP} channels produced no significant effects on cell volume regulation. The former observation is in contrast to an earlier report that Ca^{2+} influx during hypotonic cell swelling may be required for activation of $I_{Cl.vol}$ in chick cardiac myocytes (508). Clemo and Baumgarten (55) have also recently directly measured membrane currents in isolated rabbit ventricular myocytes using the perforated patch technique while monitoring changes in cell volume during hypotonic cell swelling. Both Gd^{3+}-sensitive (Ba^{2+}-insensitive) inwardly rectifying cation selective currents and 9-AC-sensitive outwardly rectifying Cl^- selective currents were activated during graded cell swelling induced by exposure to hyptonic solutions.

2. Basal or spontaneously active $I_{Cl.vol}$

In rabbit atrial myocytes, under presumably isotonic conditions, it was observed that the rapid activation and inactivation of the transient outward K^+ current (I_{to1}) was followed by a sustained tetraethylammonium-, Ba^{2+}-, and Cs^+-insensitive current (I_{sus}), which was Cl^- sensitive, outwardly rectifying, and blocked by SITS and DIDS (97). This sustained current was basally active in the absence of exogenous stimulation of cAMP, cytosolic Ca^{2+}, or cell swelling and thus was initially identified as a novel Cl^- conductance. A similar basally active Cl^- current was also observed in cultured chick cardiac myocytes (264). However, subsequent experiments in rabbit atrial myocytes (92) with more careful control of solution osmolarities and monitoring of cell volume showed that the basally active Cl^- current ($I_{Cl.b}$) under isotonic conditions (294 ± 3 mosM) could be further augmented by extracellular hypotonicity (217 ± 2 mosM)-induced cell swelling (140 ± 15% increase in cell volume) and inhibited by extracellular hypertonicity (361 ± 3 mosM)-induced cell shrinkage (53 ± 3% decease in cell volume), suggesting that $I_{Cl.b}$ in rabbit atrial myocytes may be due to the same ion channel as $I_{Cl.vol}$. Both $I_{Cl.b}$ and $I_{Cl.vol}$ in rabbit atrial myocytes were Cl^- dependent, outwardly rectifying under symmetrical Cl^- conditions, volume sensitive, inhibited by disulfonic stilbenes, and suppressed by α_{1A}-adrenergic stimulation of PKC (92). $I_{Cl.b}$ with pharmacological and anion-selective properties similar to $I_{Cl.vol}$ was also observed in canine ventricular myocytes (445). Subsequent detailed single-channel studies in rabbit myocytes (93) confirmed $I_{Cl.b}$ and $I_{Cl.vol}$ are likely mediated by the same unitary channel (see sect. II*C3*).

$I_{Cl.swell}$ is conventionally considered to be activated only by cell swelling and to be inactive under normal physiological isotonic conditions. It is possible, however, that such channels are active over a range of volume states, which includes basal isotonic conditions, and therefore may play a role even in the absence of pathological cell swelling. In fact, even under isotonic conditions, volume constancy of any mammalian cell is continuously challenged by the transport of osmotically active substances across the cell membrane and alterations in cellular osmolarity by metabolism (239). It is conceivable that the operation of cell volume regulatory mechanisms, including $I_{Cl.vol}$, are able to sense not only hypotonicity and hypertonicity but also isotonicity, and thus continuously adjust their activities contributing to overall cell volume homeostasis. On the other hand, the cell isolation procedure and experimental manipulations may cause $I_{Cl.vol}$ to be recorded under isotonic conditions, despite a lack of detectable activity under physiological conditions in vivo. In canine atrial myocytes, $I_{Cl.vol}$ was spontaneously activated even under isotonic conditions (400). There is certainly precedence for spontaneous activation of $I_{Cl.vol}$ under presumably isotonic conditions in a variety of different types of small cells subjected to the ruptured patch-clamp technique (83, 268, 314). Factors other than the difference in osmotic strength of the internal and external solutions that might contribute to changes in cell volume during ruptured patch-clamp experiments include the hydrostatic pressure difference between the pipette and the cell interior and the relative rate of fluid flow across the cell membrane in relation to the rate of flow between the pipette and the cytosol (450). Recent evidence suggests that persistent activation of $I_{Cl.vol}$ may occur in certain myocardial pathological conditions (see sect. V).

3. Unitary currents

There exists some uncertainty with regard to the identification of unitary currents associated with $I_{Cl.vol}$ in most mammalian cells (see Refs. 313, 317, 412 for review). In some mammalian cells, stationary noise analysis of macroscopic currents estimated a small single-channel conductance of 0.1–2 pS, whereas a number of other studies in epithelial cells, osteoblasts, glial cells, and muscle cells identified intermediate (20–90 pS) conductance outwardly rectifying single channels responsible for $I_{Cl.vol}$. It has recently been suggested (412) that the apparent discrepancy between the conductances estimated from noise analysis and those from direct single-channel measurements might be attributed to the fact that cell

swelling rather than causing graded increases in P_o (an assumption of noise analysis), instead appears to involve changes in the number of active channels with a fixed P_o (197, 397).

In heart, an early study provided evidence that large-conductance (~400 pS) channels may be responsible for $I_{Cl.vol}$ in cultured neonatal rat ventricular myocytes (68). Spontaneous single-channel activity was recorded in both excised inside-out and cell-attached membrane patches, and similar channel activity could be elicited in some patches by exposure to hypotonic media. Channels showed multiple subconductance states, voltage-dependent inactivation, relatively high selectivity to Cl^- over Na^+, and block by SITS. However, failure to record similar single-channel activity in freshly isolated rat myocytes, regardless of the age of the animal and the recording patch configuration used, suggests that the appearance of these channels may be somehow dependent on cell culture conditions. Similar channels have yet to be reported for acutely isolated adult myocytes, raising doubts about whether similar channels are normally responsible for macroscopic $I_{Cl.vol}$.

Unitary currents that might be responsible for macroscopic $I_{Cl.vol}$ in adult mammalian cardiac myocytes have been difficult to detect. For example, in inside-out patches derived from rabbit atrial myocytes, outwardly rectifying Cl^- channels (ORCC) with an intermediate unitary conductance of ~60 pS (in symmetrical Cl^-) were detected in only 9% of a total of 155 patches examined under basal, unstimulated, conditions (94), and SITS and DIDS induced a strong but reversible type of flicker block. It was suggested that these ORCC might be responsible for the basal or spontaneously active component of macroscopic I_{Cl} previously described in these cells by the same investigators (97). The relationship between these basally active channels and $I_{Cl.vol}$ was examined in a later study (93) that directly compared the properties of unitary ORCC in cell-attached membrane patches from myocytes exposed to isotonic and hypotonic solutions. Very similar ORCC with a unitary conductance of ~28 pS were observed under isotonic and hypotonic conditions. Active channels were more prevalent in patches from cells exposed to hypotonic solutions but exhibited approximately the same P_o (0.67) under the two conditions. In addition, ORCC recorded under the two conditions exhibited similarities in unitary conductance, rectification, and block by tamoxifen as well as similar kinetic properties (minimal 3 open and 4 closed state kinetic model). These results are consistent with those reported for ORCC and $I_{Cl.vol}$ in noncardiac cells (197, 317, 412).

Because of the limited number of studies that have attempted to measure unitary currents responsible for $I_{Cl.vol}$ in the heart, it is difficult to predict whether or not $I_{Cl.vol}$ is uniformly expressed with similar properties in all types of mammalian cardiac cells and is necessarily generated by the same population of ORCC channels as described in rabbit atrial myocytes (97). A preliminary report from cultured chick myocytes suggested that a similar channel may underly $I_{Cl.vol}$ in that tissue (511), but further studies are required to establish the generality of these results. In light of some of the different and unusual properties that have been described for macroscopic $I_{Cl.vol}$ in canine atrial myocytes compared with other types of cardiac (and noncardiac) cells, it would seem especially useful to identify the nature the unitary currents responsible for $I_{Cl.vol}$ in that tissue and to validate whether or not these channels are, in fact, modulated by PKA, cAMP, and PKC (see sect. II*C4*).

A recent report (371) describes the properties of unitary Cl^- currents activated by positive pipette pressure to outside-out membrane patches from human atrial myocytes. Surprisingly, these channels were voltage independent, DIDS and 9-AC sensitive, exhibited a conductance of ~9 pS, and had a linear current-voltage relationship with symmetrical Cl^-. Despite the fact that these channels were reportedly not activated or modulated by forskolin, cAMP, or isoproterenol, the conductance and rectification properties of these channels more closely resemble the characteristic single-channel and macroscopic properties of $I_{Cl.PKA}$ (102, 136) rather than $I_{Cl.vol}$. It is not certain whether or not changes in membrane patch hydrostatic pressure necessarily activate the same type of channels as cell swelling induced by hypotonic solutions, since $I_{Cl.vol}$ consistently exhibits strong outward rectification, even in symmetrical Cl^-. It is noteworthy that the stretch-activated I_{Cl} induced by whole cell inflation of rabbit atrial and SA nodal cells (153) also exhibited a linear current-voltage relationship in symmetrical Cl^-. Obviously, additional studies are required to determine whether or not these different types of stimuli activate the same population of Cl^- channels in cardiac myocytes. It also is not clear at this time whether or not cAMP-independent activation of CFTR Cl^- channels by pressure- or cell volume-induced alterations in the actin cytoskeleton (337) might also contribute to some of the macroscopic or unitary currents measured under these conditions.

4. Regulation by phosphorylation

In most cells, activation of $I_{Cl.vol}$ does not appear to require phosphorylation, since channels can be activated in the absence of cytoplasmic Mg^{2+} and in the presence of nonhydrolyzable analogs of ATP (313, 317, 412). Although phosphorylation by protein kinases does not appear to play a direct role in channel activation, they may modulate channel activity by direct phosphorylation of the channel or some accessory protein that regulates channel activity. In most cells, there appears to be little, if any, evidence that $I_{Cl.vol}$ is regulated by PKA phosphorylation. However, the role of PKA phosphorylation of $I_{Cl.vol}$ in heart is controversial. In canine ventricular cells, $I_{Cl.vol}$ was reported to be insensitive

to the PKA inhibitor *N*-(2-[methylamino]ethyl)-5-isoquinolinesulfonamide (H-8) (445). In cultured chick cardiac myocytes, $I_{Cl.vol}$ has been reported to be inhibited by cAMP, forskolin, phosphodiesterase inhibitors, and phosphatase inhibitors, suggesting that activation of $I_{Cl.vol}$ during cell swelling may actually involve dephosphorylation of a PKA-dependent phosphorylation site on the channel protein (154). In marked contrast, variable effects of isoproterenol and forskolin on $I_{Cl.vol}$ have been reported in canine atrial myocytes (88), with some cells responding with stimulation, inhibition, or no response. Similar to the results obtained in cultured chick myocytes, the inhibitory effects were blocked by a PKA inhibitor; however, the stimulatory effects of isoproterenol and forskolin were insensitive to PKA inhibition (88). It was proposed, therefore, that cAMP may directly activate $I_{Cl.vol}$ in a PKA-independent fashion, analogous to the direct activation of the pacemaker current, I_f, by cAMP (82). The same group has also reported a similar stimulatory effect of forskolin on $I_{Cl.vol}$ in human atrial myocytes (327). In contrast, in guinea pig atrial and ventricular myocytes, no consistent effects attributable to cAMP on $I_{Cl.vol}$ were observed (451).

The conflicting effects of cAMP and PKA (and PKC as well) phosphorylation on $I_{Cl.vol}$ reported in canine atrial cells compared with other cardiac preparations may reflect genuine differences in the properties of the protein responsible for $I_{Cl.vol}$ between different species and compared with most noncardiac preparations in which $I_{Cl.vol}$ has been studied. However, these inconsistent effects may also be explained by difficulties that might result from studies of the dependence of $I_{Cl.vol}$ on cytoplasmic Ca^{2+} or phosphorylation pathways that are carried out in cells that may express a number of different types of Cl^- channels (in addition to $I_{Cl.vol}$) and rely solely on macroscopic current measurements (especially voltage ramps) to assess I_{Cl} function. For example, many cardiac cells that express $I_{Cl.vol}$ also express $I_{Cl.PKA}$ (CFTR), making it difficult to clearly distinguish effects of cAMP stimulation on the two channel types. This problem is accentuated when membrane currents are studied only using asymmetric Cl^- gradients (88, 89, 154, 327), since nearly all types of Cl^- channels are expected to exhibit outwardly rectifying current-voltage relations under these conditions. In the case of canine atrial cells, contamination of macroscopic $I_{Cl.vol}$ by $I_{Cl.PKA}$ is not expected to be a major source of concern, since functional as well as molecular studies fail to observe significant expression of CFTR channels in canine cardiac myocytes (185, 404). However, these cells do express Ca^{2+}-activated Cl^- channels in significant abundance (516, 518), and these channels also exhibit outward rectification in asymmetrical Cl^- but become linear in symmetrical Cl^- (64, 499; Table 1). Calcium-activated Cl^- channels would thus be expected to be activated as cytoplasmic Ca^{2+} rises in response to elevations of cAMP, or even in response to a rise in cytoplasmic Ca^{2+} that may be initiated by hypotonic cell swelling (431). Adequate resolution of the issue of whether or not $I_{Cl.vol}$ is regulated by cAMP and/or PKA phosphorylation in heart requires additional whole cell experiments in cardiac cells from a wider variety of species using symmetrical Cl^- gradients to allow clearer separation of macroscopic $I_{Cl.vol}$ (outwardly rectifying in symmetrical Cl^-) from other contaminating Cl^- currents such as $I_{Cl.PKA}$ or $I_{Cl.Ca}$ (linear current-voltage relations in symmetrical Cl^-), which may also be directly or indirectly modulated by cytoplasmic Ca^{2+} or protein kinases. It is noteworthy that cAMP has been reported to activate $I_{Cl.vol}$ in rat hepatocytes, possibly by altering the volume set point of the channels (289). However, unlike studies in human and canine atrial cells (88), this study was carried out using symmetrical Cl^- gradients, and cAMP alone was capable of activating the outwardly rectifying conductance even in the absence of hypotonic cell swelling. Another possible complicating factor might be the expression of outwardly rectifying (symmetrical Cl^-) Cl^- channels activated by cAMP, which are distinct from CFTR or $I_{Cl.vol}$ (283). Final resolution of this issue may require an examination of the effects of cAMP and PKA on the unitary currents responsible for $I_{Cl.vol}$ in heart. Unfortunately, the unitary currents responsible for $I_{Cl.vol}$ in canine atrial and ventricular myocytes have yet to be identified.

Another potentially important regulatory mechanism of $I_{Cl.vol}$ is phosphorylation by tyrosine protein kinase. It has been reported that $I_{Cl.vol}$ in canine atrial cells may be regulated by tyrosine protein kinase (402). The tyrosine kinase inhibitor genistein was found to decrease the activation of $I_{Cl.vol}$ in response to hyptonic cell swelling, an effect prevented by thiophosphorylation using adenosine 5′-*O*-(3-thiotriphosphate) (ATPγS), but not mimicked by the inactive analog daidzein. Again, asymmetrical Cl^- gradients were used in this study, making it difficult to distinguish effects of these agents on macroscopic $I_{Cl.vol}$ from possible effects on other types of Cl^- channels, which all exhibit similar outwardly rectifying current-voltage relations under these experimental conditions. The most obvious difficulty in studying the regulatory role of tyrosine protein kinases is the general lack of specific pharmacological tools to assess function. In the case of genistein, inhibitory effects on serine/threonine protein phosphatases have been suggested (193, 347). Recent studies of the effects of genistein on CFTR Cl^- channels suggest that this compound may directly interact with CFTR possibly competing with ATP at one of the NBD (126, 467).

Although in most extracardiac mammalian cells there appears to be little evidence supporting an important regulatory role of tyrosine protein kinase on $I_{Cl.vol}$ (313, 317), data supporting such a role have been obtained in human intestinal cells (438) and more recently in bovine endothelial cells (460). However, in these studies, it

is not possible to conclude whether or not the substrate for tyrosine phosphorylation is the channel itself or another regulatory protein. Subsequent studies have suggested that tyrosine kinase phosphorylation may result in activation of the Rho-Rho kinase pathway, with alterations in the actin cytoskeleton possibly mediating changes in $I_{Cl.vol}$ (315, 437). Further studies are needed to more clearly establish the exact role or tyrosine kinase, Rho-Rho kinase, and cytoskeletal rearrangements in the regulation of $I_{Cl.vol}$ in mammalian cells.

A novel regulatory mechanism linking α-adrenoceptor activation to inhibition of $I_{Cl.vol}$ in rabbit atrial myocytes was characterized (92). With the use of symmetrical Cl^- solutions to effectively separate macroscopic $I_{Cl.vol}$ from other Cl^- currents such as $I_{Cl.PKA}$ or $I_{Cl.Ca}$, it was shown that the inhibitory effects of α-adrenoceptor activation on $I_{Cl.vol}$, activated by hypotonic cell swelling, were prevented by nonspecific protein kinase inhibitors like staurosporine and H-7 and the specific PKC inhibitor bisindolylmaleimide. Furthermore, the inhibitory effects of α-adrenoceptor stimulation on $I_{Cl.vol}$ were mimicked by phorbol esters and prevented by prolonged phorbol ester-induced downregulation of endogenous PKC activity. The coupling of α-adrenoceptor activation to PKC-induced inhibition of $I_{Cl.vol}$ was suggested to be mediated by a pertussis toxin-sensitive G protein. A similar inhibitory effect of PKC on $I_{Cl.vol}$ in guinea pig atrial and ventricular myocytes and canine ventricular myocytes has recently been confirmed (57, 91). In contrast, the same laboratory that has reported that PKA activates $I_{Cl.vol}$ in canine atrial myocytes (88) has also recently suggested that PKC activation may stimulate $I_{Cl.vol}$ in canine atrial cells (89). Native $I_{Cl.vol}$ in extracardiac mammalian cells has been reported to be activated (356), inhibited (61, 80), or not affected by PKC activation (234, 318).

Finally, the possible role of Ca^{2+}/calmodulin-dependent protein kinase in the regulation of $I_{Cl.vol}$ in heart has yet to be tested. There is limited evidence that Ca^{2+}/calmodulin-dependent protein kinase may regulate volume-activated iodide and taurine fluxes in HeLa cells (227).

5. ClC-3: a new molecular candidate for $I_{Cl.vol}$

Over the past few years, several different gene products have been proposed to be responsible for $I_{Cl.vol}$, including P-glycoprotein (P-gp) and pI_{Cln}. However, it now seems likely that these proteins may not encode $I_{Cl.vol}$ but instead may regulate endogenous $I_{Cl.vol}$ (see sect. III). This highlights a particular problem in studying the molecular form of this channel. Most mammalian cell lines express an endogenous form of $I_{Cl.vol}$. Therefore, it is essential that membrane currents due to transgenic expression be easily separated from the endogenous $I_{Cl.vol}$. This can be accomplished by picking an expression cell line with a low density of endogenous volume-regulated Cl^- channels so that currents due to transgenic expression can be easily recognized. Alternatively, mutational alterations in the functional properties of the expressed channel protein, or the use of antisense oligonucleotides, can be used to distinguish transgenic from endogenous channels. Unfortunately, even these techniques are apparently not always completely definitive (148, 333, 447).

ClC genes encompass a large family of gene products that, when expressed, function as voltage-dependent anion channels (205, 206). Expression of ClC-2 has been shown to yield volume-sensitive Cl^- channels, which are inwardly rectifying, and have an anion selectivity of $Cl^- \geq Br^- > I^-$ (147, 210). These characteristics contrast to the typical properties of $I_{Cl.vol}$ found in most native mammalian cells, which exhibit outward rectification and an anion selectivity of $I^- > NO_3^- > Br^- > Cl^-$ (313, 317, 412). However, outwardly rectifying anion channels with such an anion selectivity have been attributed to another member of the ClC family, ClC-3, originally cloned from rat kidney and expressed in oocytes and mammalian cells (219, 220). These properties along with the demonstration that the unitary currents are intermediate-conductance ORCC that are strongly inhibited by PKC suggested that ClC-3 may be a potentially interesting molecular candidate for $I_{Cl.vol}$ in heart and other mammalian cells.

A full-length ClC-3 cDNA was recently cloned (95) from guinea pig ventricle (gpClC-3) that had 91.5% nucleotide sequence homology and 98.4% amino acid sequence identity with rat kidney ClC-3 (rClC-3; Ref. 220). Stable or transient transfection of gpClC-3 into NIH/3T3 cells yielded a basally active Cl^- conductance that was strongly modulated by cell volume. Many properties of the expressed $I_{gpClC-3}$ resemble those reported for native $I_{Cl.vol}$ in heart and other tissues, including an outwardly rectifying unitary slope conductance of 40 pS, an anion selectivity of $I^- > Cl^- > Asp^-$, inactivation at positive potentials, increase by extracellular hypotonicity, and inhibition by hypertonicity, by extracellular nucleotides, by phorbol esters, by stilbene derivatives, and by tamoxifen. Expressed $I_{gpClC-3}$ could be separated from the small endogenous $I_{Cl.vol}$ known to be present in untransfected NIH/3T3 cells (268) by a 16- to 30-fold higher current density. In addition, site-directed mutagenesis of an asparagine near the end of the transmembrane spanning domains (N579K, see Fig. 6*A*) altered rectification and anion selectivity of the expressed $I_{gpClC-3}$. It has recently been confirmed that expression of the human homolog, hClC-3, in mammalian fibroblasts gives rise to similar cell volume-regulated Cl^- currents (171) as has been reported for gpClC-3. The ClC-3 gene product, which has recently been shown to be expressed in vascular and visceral smooth muscle cells as well (80, 498), may be responsible for the native $I_{Cl.vol}$ present in these tissues and may play a role in the generation of myogenic tone (308, 309).

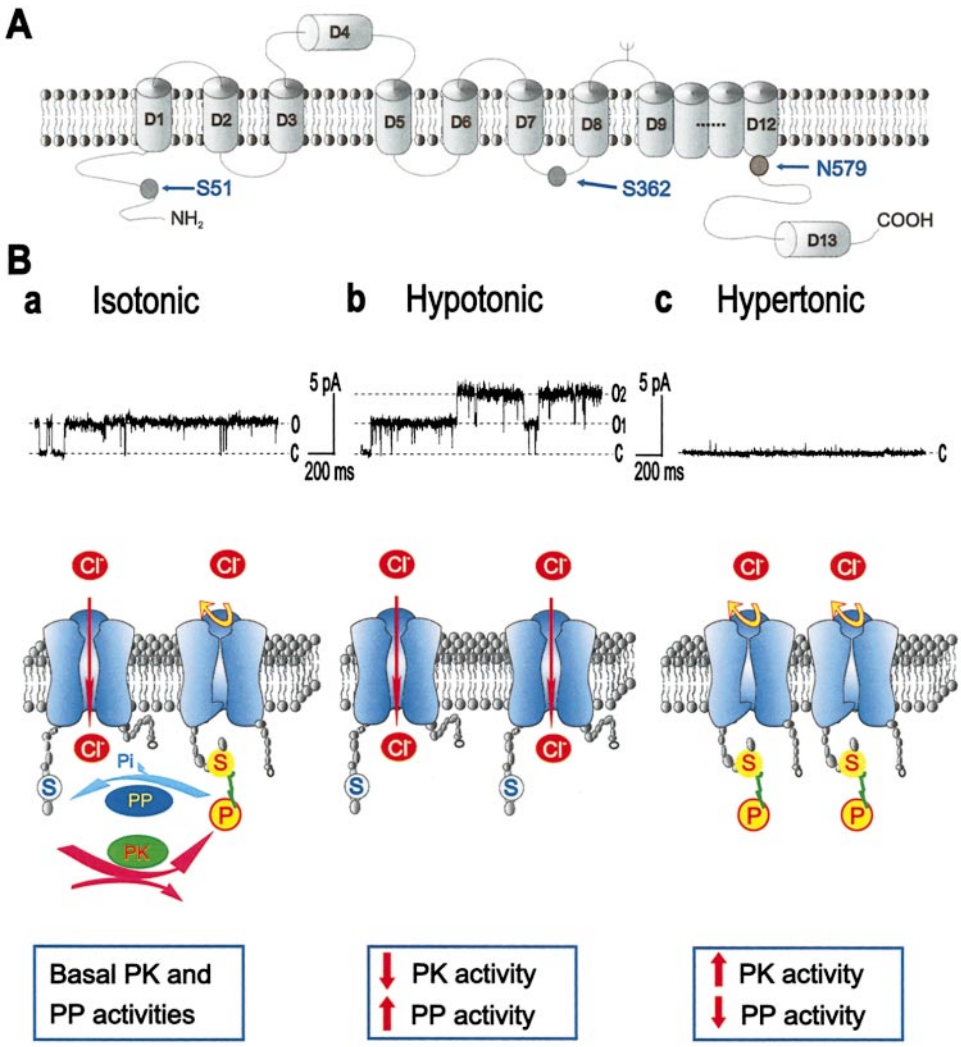


FIG. 6. Predicted membrane topology of ClC-3 (*A*) and model of ClC-3 regulation by cell volume involving protein kinase phosphorylation and dephosphorylation (*B*). Membrane topology model is based on topological model of human ClC-1 (372). Recent analysis of methanethiolsulfonate (MTS) reagent accessibility of cysteine-substituted residues in the D3-D5 regions indicates that D4 may loop back into the membrane and, along with residues in D3 and D5, form part of the ClC pore (P1) region (110). However, changing neutral asparagine at 579 to positively charged lysine (N579K) altered anion selectivity and rectification of guinea pig ClC-3 channels expressed in NIH/3T3 cells (95); corresponding amino acids in ClC-0 (343) and ClC-2 (210) have also been implicated in anion selectivity and rectification properties. Serine-51 near the NH_2 terminus and serine-362 in the D7-D8 linker represent the 2 primary cytoplasmic consensus PKC phosphorylation sites. In model in *B*, ClC-3 channels may exist in either an active dephosphorylated state or a closed phosphorylated state. Gating of channel is controlled by a volume-sensitive phosphorylation-dephosphorylation cycle mediated by a protein kinase (PK) and protein phosphatases (PP). Conformational changes in ClC-3 due to phosphorylation of serine-51 close channel, whereas conformational changes due to dephosphorylation of serine-51 cause channels to open. Under isotonic conditions (*a*), basal PK and PP activities prevent most ClC-3 channels from opening, and only a few channels are dephosphorylated. These few dephosphorylated active channels generate a "basal" current (one channel opening when recorded in a cell-attached patch shown above). Under hypotonic conditions (*b*), PK activity is diminished due possibly to dilution, redistribution, and reduced expression of PK and/or increase in PP activity, ClC-3 channels are dephosphorylated, and more channels open (2 open channels in the same cell-attached patch shown above) which results in a larger macroscopic current. Under hypertonic conditions (*c*), PK activity is increased and/or PP activity is diminished and more channels close (no open channels in the cell-attached patch shown above). [Model based on experimental results obtained in Duan et al. (91).]

6. *Molecular mechanism of ClC-3 regulation by cell volume*

A consistent feature of $I_{Cl.vol}$ observed in native cardiac cells is a temporal lag between the onset of cell swelling and detectable activation of $I_{Cl.vol}$ (154, 390, 402, 403, 451), suggesting that some metabolic or enzymatic intermediate may play a role in coupling changes in cell volume to $I_{Cl.vol}$ activation. In fact, in cultured chick cardiac myocytes, a dynamic balance between kinase (PKA) and phosphatase activity was previously suggested to be

a primary transduction process responsible for activation of $I_{Cl.vol}$ (154). A prominent feature of $I_{gpClC-3}$ (95), I_{rClC-3} (220), native $I_{Cl.vol}$ in guinea pig (91) and rabbit cardiac myocytes (92) and some other mammalian cells (61, 80, 461) is its sensitivity to inhibition by stimulation of PKC.

A recent study (91) directly tested the hypothesis that the PKC phosphorylation of ClC-3 may represent an important molecular link between changes in cell volume and channel regulation. Hypotonic cell swelling was shown to activate, whereas hypertonic cell shrinkage was shown to deactivate I_{ClC-3} expressed in NIH/3T3 cells and $I_{Cl.vol}$ in native guinea pig atrial and ventricular myocytes, effects that could be mimicked under isotonic conditions by inhibition and stimulation of endogenous PKC, respectively. Moreover, phosphatase inhibitors such as okadaic acid and calyculin A also inhibited I_{ClC-3}. These results indicate that an important regulatory mechanism modulating ClC-3 activity is its phosphorylation state. Of the two primary PKC phosphorylation sites predicted to be intracellular on ClC-3 (see Fig. 6*A*), mutation of one of them in the NH_2-terminal region, serine-51, to alanine (S51A) completely eliminated the response of I_{ClC-3} to PKC activation, phosphatase inhibition, and cell swelling. Mutation of S351A, positioned between transmembrane segments D7 and D8, had an intermediate effect. Thus one possible model (Fig. 6*B*) for the link between cell volume changes and PKC-dependent phosphorylation of ClC-3 may involve the translocation of PKC (and/or protein phosphatases) to and from the vicinity of the channel. Translocation of PKC away from the channel during cell swelling would allow the phosphorylation/dephosphorylation equilibrium of the channel to favor dephosphorylation and channel opening, whereas activation of PKC, not in the vicinity of the channel, might have no effect on channel activity. Therefore, the phosphorylation/dephosphorylation equilibrium of the population of $I_{Cl.vol}$ channels in a cell and the number of kinase proteins (or phosphatases) translocated in response to cell swelling might determine the overall response of $I_{Cl.vol}$ to changes in cell volume. This translocation could be very subtle and occur in close proximity to the membrane and could involve the cytoskeleton (222). The activity of PKC (241), as well as other kinases (462), has been shown to be modulated by acute changes in cell volume (for review, see Ref. 239).

As illustrated in Figure 6*B*, under isotonic conditions (*a*), a balance of basal protein kinase (PK) and protein phosphatase (PP) activities may maintain most ClC-3 channels in a phosphorylated, closed state and only a few channels reside in a dephosphorylated, open state. These few active channels would generate a "basal" current ($I_{Cl.b}$). With exposure to hypotonic conditions and subsequent cell swelling (Fig. 6*Bb*), PK activity may be diminished due possibly to dilution (293), redistribution, or alteration (272, 328, 462), or cell swelling may alter PP activity (203). Under these conditions, dephosphorylation of ClC-3 causes more channels to open, producing a larger macroscopic current. With hypertonic cell shrinkage (Fig. 6*Bc*), PK activity may be increased (85) and/or PP activity may be diminished, causing more channels to become phosphorylated and close. Thus serine-51 in the PKC phosphorylation site near the NH_2 terminus of ClC-3 may represent an important volume sensor of the channel that directly links channel gating to alterations in intracellular PK-PP activities. The volume sensor may be continuously regulated by cell volume, although the details of how PK and PP activities are regulated by cell volume needs further elucidation. Exactly how phosphorylation of serine-51 is translated into a change in the number of functional channels is not understood. It may involve a simple conformational change in the protein (as suggested in Fig. 6*B*) or the NH_2 terminus of ClC-3 may form an inactivation "ball," and phosphorylation of serine-51 at the NH_2 terminus by PK may be essential for the ball to fit its "receptor" possibly near the inner mouth of the pore, in a manner analogous to the N-type or "ball-and-chain" inactivation mechanism characteristic of K^+ channels (183) and proposed for ClC-2 channels (147; see also Ref. 181). The regulation of ClC-3 by phosphorylation during changes in cell volume as proposed in Figure 6*B* is very similar to the role that phosphorylation has been proposed to play in the regulation of the K^+-Cl^- cotransporter by changes in cell volume (see sect. IV).

Although these data provide strong evidence in support of ClC-3 as the gene responsible for $I_{Cl.vol}$ in some cardiac cells and suggest that the phosphorylation state of this protein may represent an important molecular mechanism linking cell volume changes to alterations in $I_{Cl.vol}$, a number of additional pieces of evidence are required to substantiate this hypothesis (319). The molecular expression of ClC-3 in different regions of the heart and in different species, including humans, needs to be carefully assessed. Verification that ClC-3 is indeed expressed in the sarcolemma should be obtained using immunohistochemical or other techniques. Biochemical verification that ClC-3 is phosphorylated in vitro has yet to be demonstrated, and whether this phosphorylation is directly affected by cell volume changes and related to changes in PK (or PP) activity or localization is presently unknown. The dependence of ClC-3 on intracellular ATP and possible permeation by organic osmolytes (155, 247, 253) should be tested. From a more mechanistic standpoint, it would be helpful to gain a better understanding of how phosphorylation of the NH_2 terminus serine-51 is translated structurally into alterations in ClC-3 channel function. An explanation of the disparate responses of native $I_{Cl.vol}$ to PKC activation reported in different cardiac and noncardiac cell types (62, 80, 89, 92, 318, 356) is also needed (see Ref. 411). Possible explanations might include *1*) the molecular form of $I_{Cl.vol}$ is different between

cell types; *2*) an additional component or subunit of the channel may be responsible for the differences, since ClC channels have been demonstrated to be capable of forming heteromultimers with distinct channel properties (267); and *3*) phosphorylation/dephosphorylation equilibria or pathways may vary in different cell types. Finally, the possible role of changes in ionic strength (106) or tyrosine phosphorylation (402, 460) in the regulation of ClC-3 channels should also be assessed.

7. Sensitivity to Cl^- channel blockers

$I_{Cl.vol}$ in cardiac cells, like in many other types of cells (317, 412), is blocked by millimolar concentrations of the stilbene derivatives SITS, DIDS, and DNDS (97, 153, 400, 401, 445, 451). The block by these compounds is usually voltage dependent, with outward currents more effectively inhibited compared with inward currents (153, 401, 451). $I_{Cl.vol}$ is also blocked less potently by carboxylic acid derivatives such as 9-AC and DPC (55, 153, 401, 451, 512). The most extensive characterization of the sensitivity of $I_{Cl.vol}$ in heart to inhibitors was made by Sorota (401) in canine atrial myocytes. Niflumic acid (100 μM), NPPB (10–40 μM), and IAA-94 (100 μM) produced complete block of $I_{Cl.vol}$; 9-AC (1 mM) and dideoxyforskolin (100 μM) produced only partial block; and DIDS (100 μM) and DNDS (5 mM) blocked outward currents more effectively than inward currents. At these concentrations, other nonspecific effects of niflumic acid, IAA-94, and NPPB were found, emphasizing that most of these compounds fall short of being considered selective antagonists of $I_{Cl.vol}$. One of the most potent inhibitors of $I_{Cl.vol}$ is the antiestrogen compound tamoxifen, which at 10 μM has been shown to nearly completely block $I_{Cl.vol}$ in NIH/3T3 fibroblasts (104), T84 colonic carinoma cells (448), and $I_{Cl.vol}$ in guinea pig atrial and ventricular myocytes (451). Although these effects of tamoxifen seemed selective for $I_{Cl.vol}$ over $I_{Cl.PKA}$ (448, 451), other possible nonselective actions of this compound have yet to be rigorously tested. Tamoxifen (10 μM) has also been shown to block the ORCC unitary currents associated with basally active and hypotonically induced $I_{Cl.vol}$ in rabbit atrial myocytes (93).

The K_{ATP} channel inhibitor glibenclamide, which has been shown to inhibit both epithelial (383) and cardiac CFTR Cl^- channels (439), also appears to significantly inhibit epithelial (265) as well as cardiac $I_{Cl.vol}$ in a voltage-dependent and reversible fashion with an estimated EC_{50} of ~60–200 μM (365, 499). It has been suggested that the ability of glibenclamide and other sulfonylurea compounds to inhibit CFTR may be due to binding of these compounds to an intracellular ATP binding site that has molecular homology to the ATP binding site in K_{ATP} channels (383). Sakaguchi et al. (365) confirmed that activation of $I_{Cl.vol}$ by hypotonic cell swelling in guinea pig atrial cells is dependent on nonhydrolyzable intracellular ATP. More recent studies have shown that sulfonylureas cause open-channel block of CFTR, implying that these compounds may bind directly in the pore (373, 382). This possibility has not yet been tested for sulfonylurea block of $I_{Cl.vol}$.

A characteristic feature of $I_{Cl.vol}$ in many noncardiac cells is inhibition by extracellular nucleotides, such as cAMP and ATP (313, 412). The block usually exhibits considerable voltage dependence, with outward currents blocked much more effectively than inward currents. Although extracellular cAMP was previously found to slightly inhibit $I_{Cl.vol}$ in canine atrial cells (401), extracellular ATP block of native $I_{Cl.vol}$ in cardiac mycoytes has been demonstrated in guinea pig atrial myocytes (91).

Finally, the pharmacological properties of expressed ClC-3 channels, which have been examined to date, seem to closely resemble those reported for native $I_{Cl.vol}$ in most mammalian cells (91, 95). Outward I_{ClC-3} is more effectively blocked by DIDS and extracellular ATP compared with inward currents, and I_{ClC-3} exhibits a similar sensitivity to block by tamoxifen as $I_{Cl.vol}$ in native cardiac cells.

8. Species and tissue distribution

The $I_{Cl.vol}$ appears to be ubiquitiously expressed in heart and has been observed in nearly every cardiac cell type examined including canine atrial and ventricular myocytes (400, 445), rabbit atrial (92) and sinoatrial myocytes (153), cultured chick myocytes (512), guinea pig atrial and ventricular myocytes (390, 451), and feline ventricular myocytes (142). Where comparisons have been made, the density of $I_{Cl.vol}$ appears to be higher in atrial myocytes compared with ventricular myocytes (400, 445, 451). Because $I_{Cl.vol}$ appears to be ubiquitiously expressed in most mammalian cells, it is likely expressed in adult rat and mouse cardiac cells as well, although this remains to be tested experimentally. $I_{Cl.vol}$ has been identified in rat neonatal myocytes (436). It is nearly unanimously agreed that $I_{Cl.vol}$ is expressed in human atrial myocytes and possibly ventricular myocytes as well (255, 327, 366, 371).

9. Physiological and pathophysiological role

A primarily physiological role of $I_{Cl.vol}$ is cell volume homeostasis. Activation of $I_{Cl.vol}$ represents one important trigger to initiate RVD (141, 239). In heart, a variety of ionic conductances have recently been shown to be modulated by changes in cell volume (450), and these all have the potential of altering electrical excitability. Because the E_{Cl} in heart is near −50 mV (see sect. IV), activation of any I_{Cl}, including $I_{Cl.vol}$, is expected to result in an increase in outward, repolarizing current during the action potential plateau and a small increase in inward, depolarizing current near the cell's resting potential (163; Fig. 8). Activation of $I_{Cl.vol}$ during cell

swelling is expected to cause a more pronounced effect on action potential duration compared with the resting membrane potential, since this current exhibits marked outward rectification. Both the action potential shortening and membrane depolarization resulting from activation of $I_{Cl.vol}$ are two effects that can accelerate the development of reentry arrhythmias (174). Such changes in electrical activity due to activation of $I_{Cl.vol}$ can be expected to contribute to the development of cardiac arrhythmias during ischemia-reperfusion, since cell swelling does occur under these conditions presumably as a result of increases in tissue osmolarity (204, 488). Alternatively, in cardiac hypertrophy, $I_{Cl.vol}$-induced action potential shortening may be antiarrhythmic, by antagonizing the excessive action potential prolongation usually associated with decreases in K^+ current density (23).

Although a number of reports have shown that cell swelling induces action potential shortening and membrane depolarization in a number of types of cardiac cells (see Ref. 450 for review), the role of $I_{Cl.vol}$ in these electrical changes has only recently been assessed. In guinea pig ventricular myocytes, cell swelling induced by hypotonic (0.67 T) solutions caused a small membrane depolarization of some 4–5 mV and an initial brief lengthening of action potential duration, followed by action potential shortening (449). In most cells, the depolarization and action potential shortening were partially prevented by DIDS, thus implicating $I_{Cl.vol}$. Swelling of dog atrial cells by inflation (positive pipette hydrostatic pressure) caused a larger membrane depolarization of some 15–20 mV that was antagonized by niflumic acid and accentuated by replacement of external Cl^- with the less permeant anion aspartate (87). The different magnitude of membrane depolarization observed in response to cell swelling of guinea pig and dog atrial myocytes might be due to differences in the density of $I_{Cl.vol}$ in the two cell types or may be due to the fact that the two different methods of initiating cell swelling differentially activate $I_{Cl.vol}$. Although activation of $I_{Cl.vol}$ normally only exerts small effects near the resting membrane potential, due to the predominence of the background resting K^+ conductance, with cell swelling the resting K^+ conductance may decrease due to dilution of $[K^+]_i$ (6, 87). This situation is nearly analogous to the accentuated effect of activation of $I_{Cl.PKA}$ on resting membrane potential, when the background K^+ conductance is reduced by reduction of $[K^+]_o$ (496). Because the background K^+ conductance may be smaller in nodal cells, membrane depolarization due to activation of $I_{Cl.vol}$ may be more prominent. The chronotropic response to mechanical stretch of mammalian sinoatrial nodal tissue has been reported to be attentuated by the stilbene derivatives DNDS, SITS, and DIDS, implicating involvement of a stretch-activated anion current, possibly similar to $I_{Cl.vol}$ (12).

D. Cl^- Channels Activated by Cytoplasmic Ca^{2+}

The transient outward current (I_{to}), activated during membrane depolarization, has been studied extensively for many years and was initially referred to as the "early outward current," "initial outward current," or "positive dynamic current" (186). The transient or rapidly activating and inactivating kinetics dictate the role that I_{to} plays in modulating cardiac electrical activity. Rapid activation of I_{to}, following Na^+ and Ca^{2+} channel activation during the action potential upstroke, induces the initial (phase 1) repolarization. I_{to} also influences the plateau phase of the action potential and terminal (phase 3) repolarization in accordance with its inactivation kinetics. Early evidence that Cl^- may be a charge carrier for I_{to} in the heart was complicated by poor voltage-clamp control of multicellular preparations, difficulties in effectively separating Cl^--sensitive components of current from overlapping K^+ currents, and complicating effects of Cl^- substitutes on intracellular Ca^{2+} activity (see Ref. 186 for review). Using 4-aminopyridine (4-AP), a blocker of K^+ current, subsequent studies concluded that I_{to} was composed of at least two components in most cardiac cells: a 4-AP sensitive, but Ca^{2+}-insensitive, K^+ current (I_{to1}) and a smaller Ca^{2+}-sensitive, 4-AP-insensitive current (I_{to2}) (173, 223, 224, 446). The molecular identification of the K^+ channel subunit(s) responsible for I_{to1} has been the subject of intense recent interest (see Refs. 16, 73 for review).

1. Macroscopic currents

A reexamination of cardiac whole cell currents revealed that I_{to2} is due to the activation of Ca^{2+}-activated Cl^- channels in many tissues. Convincing evidence of Ca^{2+}-activated Cl^- currents resembling I_{to2} was first reported in rabbit ventricular myocytes (517). The residual transient outward current remaining in the presence of millimolar concentrations of 4-AP was selective for Cl^-. Although measurements of SITS-sensitive tail currents were hampered by rundown of I_{Ca}, currents were shown to reverse close to E_{Cl}. Subsequent studies in rabbit atrial myocytes and dog ventricular myocytes showed more clearly that these channels reversed close to E_{Cl} and exhibited Cl^- gradient-dependent rectification properties (516, 518). The Ca^{2+}-dependent current was still present after replacing K^+ with Cs^- and was reduced in low internal or external Cl^-. Activation of the Cl^- current required Ca^{2+} current activation, and blocking I_{Ca} with nisoldipine or Cd^{2+} abolished I_{to2}. Conversely, increasing I_{Ca} with the β-adrenergic agonist isoproterenol augmented I_{to2}. Calcium-induced Ca^{2+} release (CICR) from sarcoplasmic reticulum (SR) is normally required for activation of $I_{Cl.Ca}$, since it is abolished upon exposing cells to caffeine or pretreatment of cells with ryanodine, suggesting that Ca^{2+} entry via Ca^{2+} channels alone is insufficient to cause activation (393, 516).

2. Gating and kinetics

Defined as SITS- or DIDS-sensitive current, $I_{Cl.Ca}$ has a bell-shaped current-voltage relation. Activation occurs at potentials slightly positive to the I_{Ca} activation threshold, peaks at potentials more positive than peak I_{Ca}, and declines at potentials approaching Ca^{2+} equilibrium potential (E_{Ca}) (517, 518). Decay of the Cl^- current before the $[Ca^{2+}]_i$ transient has reached its peak has been reported in canine ventricular myocytes (446) and Purkinje cells from rabbit heart (393). It was postulated that this behavior could be attributed to either alterations in voltage-dependent Ca^{2+} influx or voltage- and calcium-dependent Ca^{2+} release from the SR (446). Alternatively, such behavior may reflect an intrinsic inactivation process associated with $I_{Cl.Ca}$ itself or may be due to the existence of subsarcolemmal Ca^{2+} gradients (393). Clearly, attempts to characterize the kinetics of activation or inactivation under these conditions are hampered by the inability to effectively study $I_{Cl.Ca}$ in isolation, with intracellular Ca^{2+} clamped, in the absence of I_{Ca} and CICR. Intrinsic voltage dependence of $I_{Cl.Ca}$ was tested more rigorously in canine ventricular myocytes by using butanedione monoxime to prevent contraction and a Ca^{2+} ionophore to effect changes in resting $[Ca^{2+}]_i$ (516). Under these conditions, with intracellular Ca^{2+} pseudo-clamped to constant levels, $I_{Cl.Ca}$ showed little or no voltage- or Ca^{2+}-induced inactivation and was essentially time and voltage independent. These and other data (217) suggest that $I_{Cl.Ca}$ behaves essentially as a ligand-gated channel, and its apparent time dependence reflects changes in intracellular Ca^{2+} in close proximity to the channels. The currents exhibit a linear current-voltage relationship with symmetrical Cl^- and have a reported anion selectivity of $SCN^- > I^- > Br^- > Cl^-$ in rabbit ventricular cells (217).

3. Unitary currents

Only one study has identified the unitary currents responsible for Ca^{2+}-activated Cl^- currents in cardiac myocytes (64); thus information on single-channel properties is rather limited. In inside-out membrane patches from canine ventricular myocytes, these channels exhibited a small single-channel conductance (1.0–1.3 pS) with several properties similar to macroscopic $I_{Cl.Ca}$ recorded from canine ventricular myocytes, including Cl^- selectivity, dependence on $[Ca^{2+}]_i$ for activation, 4-AP resistance, and block by the anion transport blockers niflumic acid and DIDS. These single channels are comparable to the low-conductance (1–3 pS) Ca^{2+}-activated Cl^- channels found in *Xenopus* oocytes (426), endocrine cells (428), cultured A6 cells (279), lacrimal gland cells (278), and smooth muscle cells (228, 452). Despite the low single-channel conductance, cardiac Ca^{2+}-activated Cl^- channels have a rather high membrane density ($\sim 3\ \mu m^{-2}$), and calculations based on estimates of cytosolic Ca^{2+} sensitivity suggest that these small-conductance Cl^- channels can contribute significant whole cell membrane currents in canine ventricular myocytes in response to changes in $[Ca^{2+}]_i$ within the physiological range (64). Ensemble averages of single-channel current recordings from inside-out patches of Ca^{2+}-activated Cl^- channels in the presence of constant bath pCa^{2+} also failed to reveal any time- or voltage-dependent gating behavior, thus confirming that cardiac $I_{Cl.Ca}$ behaves essentially as a ligand-gated channel. It is noteworthy that in some cell types, a rise in $[Ca^{2+}]_i$ is believed to not only activate Ca^{2+}-activated Cl^- channels but may also cause inactivation by activation of a Ca^{2+}-dependent protein kinase, possibly involving PKC (29) or Ca^{2+}/calmodulin-dependent protein kinase (469). The regulation of cardiac $I_{Cl.Ca}$ by protein kinases, phosphatases, G proteins, and other potential signaling pathways has not yet been examined in any detail.

The unitary Ca^{2+}-activated Cl^- channel currents characterized in inside-out membrane patches from canine ventricular mycocytes (64) exhibit a surprisingly low $[Ca^{2+}]_i$ sensitivity (dissociation constant $\sim 150\ \mu M$) compared with Ca^{2+}-activated Cl^- channels described in some other types of cells (240). This may reflect the loss of a cytosolic component required for channel activation in detached membrane patches, or it may reflect the presence of significant Ca^{2+} concentration gradients between the subsarcolemmal space and bulk cytoplasmic Ca^{2+}. The existence of subcellular intracellular Ca^{2+} gradients in cardiac cells is now well established (244, 260) and believed to play a significant role in excitation-contraction coupling (39, 410). In rat ventricular myocytes, using the Na^+/Ca^{2+} exchange current as an indicator of subsarcolemmal release of Ca^{2+} in conjunction with digital imaging techniques (442), calculations suggest that the subsarcolemmal $[Ca^{2+}]$ rises and falls more quickly and reaches a higher peak than does the bulk $[Ca^{2+}]$. Two components of $I_{Cl.Ca}$ have been described during large intracellular Ca^{2+} transients in rabbit Purkinje cells (330), which may represent two separate populations of Cl^- channels or may be related to the presence of spatial and temporal inhomogeneities of $[Ca^{2+}]_i$. Large differences in the calculated subsarcolemmal $[Ca^{2+}]$ controlling the activation of $I_{Cl.Ca}$ ($\sim 112\ \mu M$), compared with the bulk $[Ca^{2+}]$ of $\sim 3.1\ \mu M$, were described in a more recent study (441), suggesting heterogeneity of the subsarcolemmal space, with Ca^{2+}-activated Cl^- channels possibly being physically located in closer proximity to the ryanodine receptors compared with the Na^+/Ca^{2+} exchanger. Although further experiments are necessary to establish the quantitative relationship between $[Ca^{2+}]_i$ and $I_{Cl.Ca}$ in cardiac cells, these data and earlier data (217, 393, 516) demonstrating that activation of $I_{Cl.Ca}$ normally requires CICR are rather consistent with the estimated intracellular Ca^{2+} sensitivity reported for

unitary Ca^{2+}-activated Cl^- channels in isolated membrane patches (64).

4. Physiological and pathophysiological role

$I_{Cl.Ca}$ exhibits significant kinetic behavior, since its time dependence necessarily follows changes in $[Ca^{2+}]_i$ in close proximity to the channels. Like other types of Cl^- channels, $I_{Cl.Ca}$ can generate inward or outward membrane current depending on the membrane potential relative to E_{Cl}. At positive membrane potentials, after CICR, $I_{Cl.Ca}$ generates a transient outward current (I_{to2}), which along with I_{to1} will help generate the initial period of repolarization (phase 1) in many cardiac cells (cf. Fig. 8). The actual role of $I_{Cl.Ca}$ in controlling phase 1 repolarization will, however, be highly dependent on the amount of Ca^{2+} entering through voltage-dependent Ca^{2+} channels as well as the numerous factors that control SR Ca^{2+} release. $I_{Cl.Ca}$ will certainly be increased by β-adrenergic receptor stimulation and decreased by muscarinic receptor stimulation, as a direct result of the effects of these interventions on the magnitude of the intracellular Ca^{2+} transient. This may explain earlier observations that I_{to} can be modulated by β-adrenergic receptor stimulation (165, 307). It has been shown that under some conditions, $I_{Cl.Ca}$ can be activated via CICR triggered by Na^+/Ca^{2+} exchange operating in the reverse mode (236), although the physiological significance of this remains to be established.

It has been suggested that elevation of $I_{Cl.Ca}$ may serve as a negative-feedback mechanism to limit Ca^{2+} entry through voltage-dependent Ca^{2+} channels by making the initial plateau level less positive (446). The effects of $I_{Cl.Ca}$ on the canine ventricular action potential have been examined and were found to depend on the rate of early repolarization and the prominence of the action potential notch (520). Inhibition of $I_{Cl.Ca}$ elevated the plateau and slightly abbreviated action potential duration when the notch was prominent. When repolarization was prolonged and the notch was shallow, inhibition of $I_{Cl.Ca}$ elevated the notch and plateau and abbreviated action potential duration. The contribution of $I_{Cl.Ca}$ to repolarization seems to be greatest during fast heart rates (215).

A transient inward current (I_{TI}), originally characterized in cardiac Purkinje fibres exposed to toxic concentrations of digitalis (214), is believed to be responsible for the generation of oscillatory afterpotentials resulting in a variety of cardiac arrhythmias (200). Although Ca^{2+}-activated nonselective cation channels (103) and Na^+/Ca^{2+} exchange (225) were long considered the primary charge carriers responsible for I_{TI}, recent data suggest that $I_{Cl.Ca}$ may also play a significant role (see Fig. 7). Han and Ferrier (157) demonstrated that in the absence of Na^+/Ca^{2+} exchange, I_{TI} in rabbit Purkinje fibers was blocked by DIDS and SITS and exhibited a reversal potential that was sensitive to the Cl^- gradient. A similar role of $I_{Cl.Ca}$ in the generation of I_{TI} in canine (516, 519) and rabbit (238) ventricular myocytes has also been demonstrated. In contrast, $I_{Cl.Ca}$ appears to play little or no role in the generation of I_{TI} in guinea pig ventricular myocytes (394). Thus the relative contributions of nonselective cation channels, Na^+/Ca^{2+} exchange, and $I_{Cl.Ca}$ to the generation of I_{TI} appears to be species and tissue dependent. In hindsight, it is not surprising that the generation of I_{TI}, which has long been known to be associated with SR Ca^{2+} overload, might be mediated, at least in part, by Ca^{2+}-activated Cl^- channels in cells that express these channels in high density.

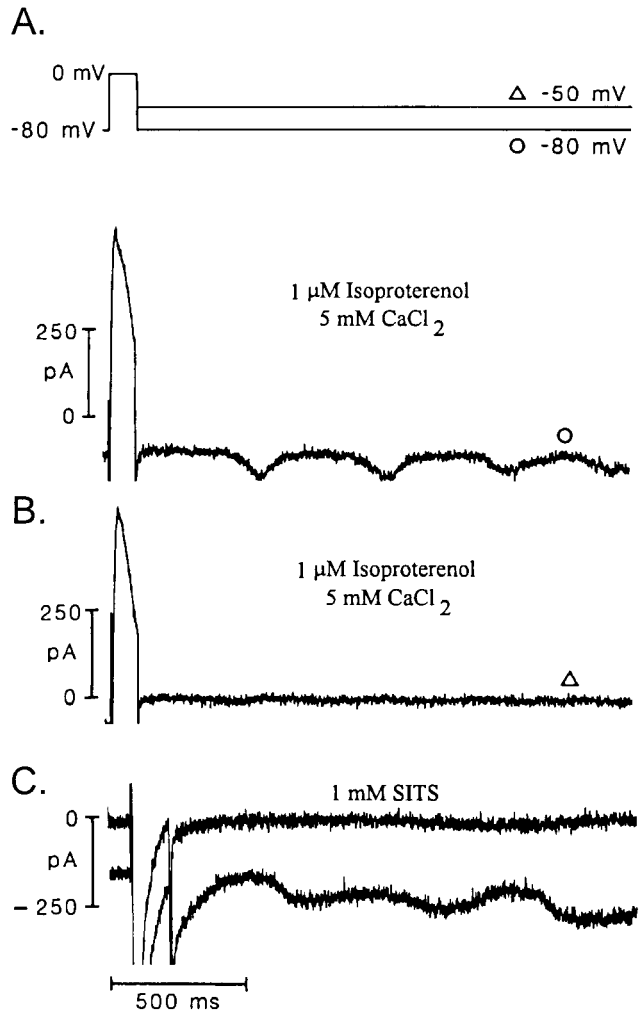


FIG. 7. Isoproterenol and high external Ca^{2+} activate Cl^-- and SITS-sensitive transient inward currents (I_{TI}) in canine ventricular myocytes. Experiment was carried out in Na^+- and K^+-free solutions. *A*: I_{TI} elicited during repolarization to −80 mV after depolarizing pulse to 0 mV in presence of isoproterenol and 5 mM external Ca^{2+}. *B*: reversal of I_{TI} during repolarization to −50 mV, estimated value of Cl^- equilibrium potential. *C*: SITS blockade of I_{TI} in a different midmyocardial cell. [Adapted from Zygmunt (516).]

5. Sensitivity to Cl^- channel blockers

$I_{Cl.Ca}$ is blocked by the disulfonic acid derivatives SITS and DIDS at concentrations (100 μM-2 mM) that reportedly do not significantly inhibit Ca^{2+} currents (217, 393, 516, 517). Both macroscopic $I_{Cl.Ca}$ and unitary Ca^{2+}-activated Cl^- channels are also blocked by niflumic acid (50 μM; Ref. 64), a reversible inhibitor (EC_{50} ~17 μM) of $I_{Cl.Ca}$ in *Xenopus* oocytes (481). Also an inhibitor of $I_{Cl.Ca}$ in *Xenopus* oocytes (491), NPPB has not been tested on $I_{Cl.Ca}$ in cardiac cells. A variety of other anionic transport inhibitors, including carboxylic acid derivatives like 9-AC and DPC, and furosemide and IAA-94, at high concentrations have been reported to inhibit $I_{Cl.Ca}$ in other types of cells including smooth muscle (240). $I_{Cl.Ca}$ in cardiac cells is also inhibited by the K_{ATP} channel inhibitor glibenclamide, with an estimated EC_{50} of ~65 μM (499).

To date, few quantitative pharmacological studies have been performed to determine the selectivity of these various anion transport inhibitors for different types of Cl^- channels (35). Future pharmacological studies in this direction will be aided considerably by advances in the molecular identification of anion channels in the various tissues and the ability to test pharmacological agents on well-defined molecular structures. Unfortunately, definitive molecular identification of the protein responsible for small-conductance Ca^{2+}-activated Cl^- channels, which seem to be most ubiquitious across different tissues and cell types, has yet to be made. A Ca^{2+}-activated Cl^- channel from bovine trachea (bCLCA1; unrelated to the ClC Cl^- channel family) has been cloned and functionally expressed in mammalian cells (70); however, its reported anion selectivity, large unitary conductance (25–30 pS), and insensitivity to niflumic acid make this an unlikely molecular candidate for the ubiquitious, small-conductance Ca^{2+}-activated Cl^- channels found in most mammalian cells. It is not known whether the properties of the recently reported truncated form of this channel (176) might more closely resemble those of native small-conductance Ca^{2+}-activated Cl^- channels. Two proteins with homology to bCLCA1 have also recently been cloned from a mouse lung cDNA library (mCLCA1; Ref. 139) and from a human genomic library (hCLCA1; Ref. 146). mCLCA1 is a 902-amino acid protein, which when expressed in HEK 293 cells gives rise to a Cl^- conductance activated by $[Ca^{2+}]_i$ and inhibited by DIDS (300 μM) and niflumic acid (100 μM). HEK cells transfected with hCLCA1 exhibit a Cl^- conductance with similar pharmacological properties and unitary currents with a slope conductance of ~13 pS. HCLCA1, however, appears to be an unlikely candidate responsible for $I_{Cl.Ca}$ in most mammalian cells, since its expression seems to be specific to intestinal epithelial and goblet cells. On the other hand, Northern analysis has shown expression of mCLCA1 in heart, lung, liver, kidney, spleen, and brain (139). This ubiquitious expression pattern and the pharmacological properties reported for expressed mCLCA1 channels make this, or another yet unidentifed member of this family, possible molecular candidates for $I_{Cl.Ca}$. However, additional evidence, including characterization of the properties of unitary currents associated with mCLCA1 expression, is needed before definitive conclusions can be made. The fact that both mCLCA1 and HCLCA1 belong to the same gene family as ECAM-1, a lung endothelial cell adhesion molecule, raises some question about their exact physiological role.

6. Species and tissue distribution

Although $I_{Cl.Ca}$ has been studied mostly in rabbit atrial, ventricular (517, 518), and Purkinje cells (393) and canine ventricular myocytes (64, 446, 499, 516), it has also been detected in sheep cardiac Purkinje fibers (223) and cultured chick cardiac cells (262). It appears to be absent in guinea pig ventricular myocytes (394). $I_{Cl.Ca}$ may also be expressed in some tissues in which a Ca^{2+}-sensitive component of I_{to} (I_{to2}) was demonstrated in earlier studies, including calf Purkinje fibers (391), elephant seal atrial fibers (284), and feline ventricular myocytes (131).

I_{to} has been measured in human atrial and ventricular myocytes and is considered one of the major repolarizing currents (230, 303, 479). The density of I_{to} in human cardiac myocytes varies in different regions (480) of the heart and changes during development (69) and disease (302). Despite the fact that early studies suggested the existence of a Ca^{2+}-sensitive component of I_{to} similar to I_{to2} in human atrial tissue (66, 107), a recent study of I_{to} in human atrial myocytes (254) failed to detect the presence of $I_{Cl.Ca}$. The 4-AP-resistant component of I_{to} detected was Ca^{2+} insensitive and attributed to voltage-dependent relief of 4-AP block of I_{to1}. Although numerous studies have demonstrated spontaneous or triggered electrical activity of excised human atrial and ventricular tissue linked to cyclic increases in SR Ca^{2+} release (67), the possible role of Ca^{2+}-activated Cl^- channels in the generation of I_{TI} in human myocardium has yet to be tested.

E. Cl^- Channels Activated by Purinergic Receptors

Extracellular ATP is known to have both positive and negative inotropic and chronotropic effects in the heart, which are species and purinergic receptor subtype dependent. Three primary purinergic mechanisms have been described in cardiac muscle (455, 456). P_1-purinergic receptor stimulation may increase K^+ conductance and inhibit β-adrenergic-stimulated adenylate cyclase, both effects mediated by a pertussis toxin-sensitive G_i protein that couples the P_1 receptor to adenylyl cyclase. P_2-purinergic receptor stimulation may enhance Ca^{2+} currents via a direct G_s protein interaction with the channel and

may stimulate phosphoinositide breakdown leading to the production of IP_3, DAG, and subsequent activation of PKC. Finally, P_3-purinergic receptor stimulation may lead to activation of the Cl^-/HCO_3^- exchanger, causing intracellular acidification and activation of a nonselective cation conductance (340). Such a simple scheme, however, is unable to account for all of the purinergic effects described in cardiac cells, and subsequent studies have revealed the exceedingly complex nature of purinergic signaling pathways as well as the complex nature of purinergic receptor subtypes (15, 473).

To date, $I_{Cl.ATP}$ represents the least-studied I_{Cl} in heart. The first evidence for the activation of I_{Cl} in response to extracellular ATP in cardiac cells was provided by Matsuura and Ehara (282) in guinea pig atrial myocytes, and only two other studies have examined the current, in rat (212) and mouse (252) ventricular myocytes. Until very recently, the properties of $I_{Cl.ATP}$ were not understood well enough to conclusively determine whether or not extracellular ATP activates a novel class of anion channels or modulates one of the other types of sarcolemmal anion channels in heart. New data (96) suggest that $I_{Cl.ATP}$ may be attributed to purinergic activation of CFTR Cl^- channels through a dual intracellular signaling pathway involving both PKA and PKC (see Fig. 1).

In guinea pig atrial cells, ATP (5–50 μM) was originally shown to activate a transient cation-selective current and a sustained time-independent Cl^- sensitive current. $I_{Cl.ATP}$ was found in ~40–50% of the myocytes tested and was also activated by extracellular ADP, AMP, and adenosine, suggesting no clear order of potency consistent with a particular purinergic receptor subtype (282). Strong buffering of $[Ca^{2+}]_i$ had little effect on $I_{Cl.ATP}$. Although it was shown that ~10% of the guinea pig atrial cells examined did exhibit $I_{Cl.PKA}$, consistent with later molecular demonstration of low-density CFTR channel expression in this tissue (198), the possible dependence of $I_{Cl.ATP}$ on the adenylyl cyclase-cAMP-PKA pathway was not directly tested in this study. In rat ventricular myocytes, ATP and ADP activated $I_{Cl.ATP}$, but AMP or adenosine had no effect, suggesting involvement of a P_2-purinergic receptor (212). The fact that $I_{Cl.ATP}$ was reported to be blocked by the stilbene derivative DIDS might be considered to provide some clue to the identity of the channel involved; however, this observation is complicated by reports that this compound may directly block P_2-purinergic receptors (32) and also blocks Cl^-/HCO_3^- exchange. These experiments also used high EGTA containing internal solutions, thus likely eliminating the possibility that the currents measured might be attributed to activation of $I_{Cl.Ca}$. The demonstration of $I_{Cl.ATP}$ in adult rat ventricular myocytes is interesting from the standpoint that this tissue normally fails to exhibit any functional $I_{Cl.PKA}$ (98), although possible involvement of the adenylyl cyclase-cAMP-PKA pathway was not tested in this study. In mouse ventricular myocytes (252), ATP and ATPγS activated $I_{Cl.ATP}$, but AMP or adenosine had no effect on the current, consistent with involvement of a P_2-purinergic receptor. In these cells, isoproterenol, forskolin, and IBMX, which caused significant stimulation of I_{Ca}, were unable to activate any detectable $I_{Cl.PKA}$. Strong buffering of $[Ca^{2+}]_i$ also did not influence activation of $I_{Cl.ATP}$, and the current-voltage relationship for $I_{Cl.ATP}$ was linear in symmetric Cl^-.

From this limited information, some tentative conclusions about the nature of $I_{Cl.ATP}$ can be advanced. All three studies above showed that buffering of $[Ca^{2+}]_i$ did not affect $I_{Cl.ATP}$, so $I_{Cl.ATP}$ cannot be attributed to activation of $I_{Cl.Ca}$, although in the absence of intracellular Ca^{2+} buffering, secondary effects on $I_{Cl.Ca}$ may be expected if purinergic stimulation causes an elevation of $[Ca^{2+}]_i$. The possible activation of $I_{Cl.vol}$ by purinergic receptor stimulation also seems unlikely, since $I_{Cl.vol}$ is expected to show strong outward rectification in symmetrical Cl^- (see sect. II*C* and Table 1), whereas the current-voltage relationship of $I_{Cl.ATP}$ appears to be linear (252). These properties of $I_{Cl.ATP}$ seem most consistent with those of $I_{Cl.PKA}$ in heart (see sect. II*A*). In this regard, it is interesting that activation of P_2-purinergic receptors in the heart has recently been shown to elevate cAMP due to activation of a specific isoform (V) of adenylyl cyclase that may be different from the isoform activated by β-adrenergic receptor stimulation (339). Thus, in some tissues, it is conceivable that activation of adenylyl cyclase V by purinergic stimulation may be preferentially linked to CFTR channels, whereas activation of adenylyl cyclase IV or VI by β-adrenergic receptor stimulation may be linked to Ca^{2+} channels and other effectors. This could explain the apparent enigma that purinergic stimulation might activate CFTR channels in some cells, in which elevations of cAMP by conventional agonists fail to activate CFTR channels.

In very recent study of mouse ventricular myocytes (96), new evidence suggests that $I_{Cl.ATP}$ may be attributed to purinergic activation of $I_{Cl.PKA}$ (CFTR) through a novel signaling pathway in this tissue. Although it was previously shown that isoproterenol, forskolin, and IBMX were unable to activate any detectable $I_{Cl.PKA}$ in mouse ventricular myocytes (252, 465), RT-PCR has now clearly confirmed expression of the mouse homolog of CFTR in heart. It was also observed that phorbol esters activate Cl^--sensitive currents very similar to the $I_{Cl.ATP}$ activated by extracellular ATP and ATPγS and that the activation of either current can be prevented by inhibition of either endogenous PKC or PKA activity. This is consistent with biochemical evidence showing that stimulation of P_2-purinergic receptors in heart leads to phosphoinositide breakdown; the production of IP_3, DAG, and subsequent activation of PKC (248, 495); as well as an elevation of cAMP (339). In addition, $I_{Cl.ATP}$, once activated by ATPγS,

could be further stimulated by isoproterenol. This is reminiscent of the known synergistic effects of PKA and PKC phosphorylation on CFTR channels (209, 290, 497; see also sect. II*B3*). Thus, in some cells, endogenous PKC activity may be low, and activation of CFTR by elevation of cAMP alone may be insufficient to activate channels (290). On the other hand, P_2-receptor stimulation would be expected to optimally activate CFTR channels due to the combined synergistic effects of PKA and PKC phosphorylation. Finally, it was shown that the properties of the unitary currents associated with activation of $I_{Cl.ATP}$ in mouse ventricular myocytes were indistinguishable from the well-known properties of CFTR unitary currents (see Fig. 5 and sect. II*A*). These data are thus most consistent with the conclusion that purinergic (P_2) receptor stimulation in heart, rather than activating a unique class of anion channels, is linked, through a dual pathway involving both PKA and PKC, to activation of CFTR Cl^- channels.

Whether or not such a scheme might also explain some of the reported difficulties in demonstrating functional CFTR channels in some cardiac tissues (e.g., rat and human) remains to be determined. Finally, the relevance to $I_{Cl.ATP}$ in cardiac tissue of recent reports (41, 414, 415) suggesting that epithelial CFTR channels can be directly activated by external ATP, in a cAMP-independent manner, is presently unclear.

F. Other ClC Cl^- Channels

On the basis of the original expression cloning of a voltage-gated Cl^- channel (ClC-0) from *Torpedo marmorata* electric organ in 1990 by Jentsch et al. (208), a new family of voltage-dependent Cl^- channels, ClC-*n*, was discovered. ClC-1 was the first member of this family discovered in mammals. Subsequently, eight more members of this family, ClC-2 (435), ClC-3 (220), ClC-4 (453), ClC-5 (117), ClC-6 and ClC-7 (30), and two kidney-specific channels (ClC-Ka and ClC-Kb) have been described (207). These channels share significant homology with ClC-0 (Fig. 6*A*), whose function as a Cl^- channel has been proven beyond reasonable doubt (120, 133, 342). ClC-0 and ClC-1 channels have been shown to form dimers (108, 292), but it is not clear whether or not all ClC channels form functional dimers or whether the "double-barreled" pore structure characteristic of ClC-0 channels (270, 291) necessarily applies to other members of this family (cf. Ref. 109).

Despite a growing body of molecular data, our present understanding of the endogenous counterparts and physiological roles of members of the ClC family are limited. ClC-1 is a major mammalian skeletal muscle Cl^- channel. Mutations in ClC-1 gene lead to myotonia (a defect in muscle relaxation) in mouse (145), goat (21), and human (229). ClC-3 encodes an outwardly rectifying PKC- and volume-regulated Cl^- current ($I_{Cl.vol}$) in various tissues (95, 220). ClC-5 is a kidney Cl^- channel, mutations of which are believed to be responsible for Dent's disease (266). Although some of these channels exhibit tissue-specific expression patterns, others show a more ubiquitious expression pattern. There presently is evidence for expression of ClC-2 (130, 435), ClC-3 (95, 220), ClC-4 (4), ClC-5 (367), ClC-6, and ClC-7 (30) in mammalian heart. These genes, therefore, represent new potential candidates for encoding Cl^- channels in sarcolemmal as well as intracellular membranes of mammalian heart. The possible formation of heterodimeric channels composed of different ClC subunits (267) potentially gives rise to an even larger variety of myocardial Cl^- channels with distinct functional properties.

Although the functional properties and physiological role of some of these channels are beginning to be characterized (e.g., ClC-0, ClC-1, ClC-2, ClC-3, and ClC-5) (see Refs. 181, 205, 207 for review), others have not yet been functionally expressed. ClC-2 has interesting functional properties and has been cloned from mammalian heart and functionally expressed in *Xenopus* oocytes. ClC-2 was orignally cloned from rat heart and brain (435), and a rabbit homolog of ClC-2 (ClC-2G) was isolated from a rabbit gastric cDNA library (275). ClC-2 is closed under resting conditions and can be slowly activated by hyperpolarization at voltages more negative than −90 mV. The instantaneous current-voltage relationship assessed by tail current analysis shows inward rectification. ClC-2 is selective for Cl^- over other anions and has an anion permeability sequence of $Cl^- = Br^- > I^-$. The channel is blocked by 9-AC, zinc, and cadmium but is largely unaffected by SITS. ClC-2 can be activated by cell swelling (147, 435) and acidic extracellular pH (210, 413). Like ClC-3 (91, 220), PKC phosphorylation of ClC-2 channels prevents its activation by cell swelling (407); however, the effects of PKA on rat ClC-2 are controversial. Protein kinase A stimulation has been reported to activate ClC-2G (385), but PKA has been reported to have little effect on ClC-2 cloned from rabbit heart and expressed in *Xenopus* oocytes (132).

The physiological role of ClC-2 channels remains uncertain because most studies have been carried out on cloned ClC-2 channels. However, endogenous Cl^- currents similar to ClC-2 have been found in a number of noncardiac cells. In neurons, ClC-2 channels have been suggested to stabilize the relationship between the membrane potential and the Cl^- equilibrium potential (53, 407), and ClC-2-like currents have been found in pancreatic acinar cells (42). Because ClC-2 is expressed in apical membranes of epithelia, it is being investigated as an alternative target for therapy in cystic fibrosis (374). A hyperpolarization-activated Cl^- current was observed in early voltage-clamp experiments in sinoatrial nodal cells

(316, 381), and native ClC-2-like inwardly rectifying anion currents have recently been identified in mouse and guinea pig atrial and ventricular myocytes (90). Considering the physiological significance of inwardly rectifying cation channels in heart (16, 81), an anionic inward rectifier ($I_{Cl.ir}$; Fig. 1 and Table 1) encoded by ClC-2 may play a significant role in some tissues and species as well.

III. PHOSPHOLEMMAN, P-GLYCOPROTEIN, AND pICln

Phospholemman (PLM) is a sarcolemmal 72-amino acid protein originally purified and sequenced from canine myocardium (329). Phosphorylation of PLM occurs after α- and β-adrenegic stimulation and correlates with increases in contractility. A similar protein has also been identified in the sarcolemma of skeletal and smooth muscle and liver cells. Despite being a major substrate for PKA and PKC phosphorylation, the exact functional role of PLM in cardiac cells remains unclear. Expression of PLM in *Xenopus* oocytes was originally reported to give rise to a novel hyperpolarization-activated I_{Cl} (298) with kinetic and rectification properties that appeared to resemble those of ClC-2 expressed channels. Mutations in a hydrophobic, putative transmembrane spanning region of PLM were reported to alter the kinetic properties of the hyperpolarization-activated I_{Cl}, suggesting that PLM may be a bonafide ion channel rather than a regulator of endogenous channels. However, a subsequent study raised doubts about this conclusion, since a similar endogenous hyperpolarization-activated I_{Cl} was observed in control oocytes (232). In further studies to test whether or not PLM forms anion-conducting channels, recombinant PLM was immunoaffinity purified from baculovirus-infected Sf21 cells and incorporated into planar lipid bilayers (296). Incorporation of PLM was associated with the appearance of ~700-pS linear conductance channels that exhibited a cation to Cl^- permeability of ~0.3 and an anion permeability of taurine $>>> NO_3^- > SCN^- \sim Cl^- > Br^-$. Furthermore, PLM was shown to exhibit distinct anion-selective and cation-selective conformations, accounting for the zwitterionic permeation properties of taurine (231). Amino acids mutations in the COOH terminus of PLM altered channel inactivation properties in bilayers (47). Although these studies provide an interesting and novel permeation model that may account for taurine transport in heart, taurine selectivity and zwitterionic permeation properties have not been confirmed for the PLM protein expressed in oocytes or any other stably transfected cell lines, raising questions related to the physiological significance of the bilayer results. Thus the question of whether or not PLM forms anion-selective channels in vivo remains open.

Moorman and Jones (297) have recently hypothesized that the major physiological role of PLM may be in cell volume regulation, by mediation of taurine efflux during RVD. Experiments examining RVD in human embryonic kidney cells suggested that PLM expression is associated with enhanced RVD during hyposmotic challenges, an effect that seemed to be reduced by incubation of cells in forskolin and IBMX, indicating regulation of PLM by PKA phosphorylation. Surprisingly, overexpression of PLM in HEK 292 cells was also found to be correlated with upregulation of endogenous $I_{Cl.vol}$ in these cells.

At this time, the physiological role of PLM remains speculative. It may, in fact, form anion-selective channels that mediate taurine efflux from cells, but further studies of PLM stably expressed in well-defined mammalian cell systems are needed to substantiate the pore-forming channel hypothesis. Phospholemman may, alternatively, coassemble with endogenous anion channel subunits, interact with endogenous anion channels to provide a specialized subtrate for PKA and PKC regulation, or play a role in the cytoskeletal control of endogenous anion channels.

P-glycoprotein, a multidrug transporter, was initially suggested to underlie $I_{Cl.vol}$ (447). This protein had promise because it was a member of the ABC superfamily of transporters of which CFTR, a known Cl^- channel, was also a member. Valverde et al. (447) reported that expression of *MDR1* (human multidrug resistance gene) coincided with expression of $I_{Cl.vol}$ activity in several mammalian cell lines. However, several studies have disputed this hypothesis (72, 104, 299, 320, 440), and it now appears likely that P-gp is not itself responsible for $I_{Cl.vol}$ (170, 484) but may regulate endogenous $I_{Cl.vol}$ in these cells. Recently, it has been proposed that P-gp may modulate the $I_{Cl.vol}$ endogenous to CHO and NIH/3T3 cells via a PKC-dependent mechanism (27). *MDR1* antisense has been shown to reduce native $I_{Cl.vol}$, increase the latency of activation of $I_{Cl.vol}$, and decrease the ability of bovine ciliary epithelial cells to volume regulate (468), effects consistent with a role of P-gp in the activation pathway for $I_{Cl.vol}$. It is not known whether P-gp may play a similar role in cardiac cells, which also express P-gp (46, 118).

Another candidate that has been proposed to underlie $I_{Cl.vol}$ is pICln. This cDNA, which is expressed in heart, encodes a 235-amino acid protein with no obvious transmembrane spanning regions. When expressed in oocytes, pICln yields a Cl^- current with many of the properties of native $I_{Cl.vol}$, particularly sensitivity to extracellular ATP (333). Unlike $I_{Cl.vol}$, pICln-expressed currents are blocked by extracellular cAMP, whereas native $I_{Cl.vol}$ is unaffected by cAMP (411). However, the report by Paulmichl et al. (333) appears to be confounded by endogenous $I_{Cl.vol}$ in oocytes (3), and it is now believed that pICln may regulate endogenous $I_{Cl.vol}$ (52, 233, 459). The exact functional role of pICln remains elusive, and whether it functions as an

anion channel-forming protein is currently being reevaluated. In cultured rat cardiac myocytes, translocation of native pICln from the cytosol to the membrane during cell swelling has been observed to correlate with taurine efflux, suggesting a role for pICln in osmolyte efflux (301). However, cell swelling apparently has no effect on pICln translocation in C6 glioma cells, in which it remains primarily localized in the cytoplasm (105). Obviously, much remains to be learned about the role of pICln in cell volume regulation.

IV. REGULATION OF INTRACELLULAR CHLORIDE CONCENTRATION

Early ion-selective microelectrode studies consistently revealed intracellular Cl^- activity (a^i_{Cl}) in mammalian Purkinje fibers and ventricular myocardial cells to be in the range of 10–20 mM (19, 37, 405, 457), higher than that (4–6 mM) predicted for passsive diffusion of Cl^- alone. These values for a^i_{Cl} place E_{Cl} normally in the range of −65 to −45 mV (see Ref. 186 for review). The accumulation of intracellular Cl^- in cardiac cells has been attributed to several electroneutral carriers or cotransporters. These include Cl^-/HCO_3^- exchange (263, 458, 494) and Na^+-dependent Cl^- transport operating as Na^+-Cl^-, Na^+-K^+-$2Cl^-$ (18, 261), and K^+-Cl^- cotransport (336). Although the first two are believed to normally mediate Cl^- uptake, the latter may function to lower a^i_{Cl}. These exchangers can be differentiated pharmacologically because Cl^-/HCO_3^- exchange is DIDS and SITS sensitive (see Fig. 3), Na^+-K^+-$2Cl^-$ cotransport is blocked by furosemide and bumetanide, chlorothiazide blocks Na^+-Cl^- cotransport, and K^+-Cl^- cotransport is blocked by high concentrations of furosemide. In addition to functioning to maintain a^i_{Cl} above its equilibrium value, Cl^-/HCO_3^- exchange also is a primary determinant of resting intracellular pH and, like the Na^+-dependent Cl^- and K^+-Cl^- cotransporters, plays a critical role in cell volume homeostasis.

Although the driving force for both Na^+-Cl^- cotransport and K^+-Cl^- cotransport are highly dependent on the respective Na^+ and K^+ gradients maintained by the Na^+-K^+ pump, the parallel operation of these two transporters is believed to result in net accumulation of intracellular Cl^-, because K^+-Cl^- cotransport is limited by relatively small changes in $[Cl^-]_i$, compared with the activity of the Na^+-Cl^- cotransporter. Thus relatively small reductions in $[Cl^-]_i$ that reduce KCl loss actually increase the driving force for Cl^- uptake by Na^+-Cl^- cotransport and Cl^-/HCO_3^- exchange (38).

An important physiological role of these electroneutral carriers is to counter passive membrane Cl^- leak and maintain a^i_{Cl} even in the presence of electrogenic Cl^- movement through the various types of sarcolemmal Cl^- channels previously described (see sect. II). A role of sarcolemmal Cl^- channels in determining a^i_{Cl} in cardiac cells is supported by the finding that β-adrenergic-induced activation of $I_{Cl.PKA}$ in quiescent guinea pig papillary muscles results in membrane depolarization that is accompanied by a significant decrease in a^i_{Cl}, measured using Cl^--selective microelectrodes (386). It seems likely that Cl^- movement through other types of sarcolemmal Cl^- channels as well will affect a^i_{Cl} in cardiac cells, but this has not yet been examined.

The most extensively characterized of the exchange transport proteins is the Cl^-/HCO_3^- exchanger, studied extensively in erythrocytes (201) and known as the band 3 anion exchanger (AE). The band 3 AE gene family is now known to comprise at least three members, AE1, AE2, and AE3, which have been cloned and characterized from a variety of tissues (7). AE1 proteins range from 848 to 929 amino acids in length and are composed of two primary structural domains, an NH_2-terminal cytoplasmic domain that mediates attachment to the cytoskeleton and a COOH-terminal domain that mediates Cl^-/HCO_3^- exchange. A recent topology study suggests that AE1 may be composed of 13 transmembrane segments (128). AE2 and AE3 encode proteins of some 1227–1237 amino acids. Each AE gene appears to transcribe multiple forms of mRNA, producing variant peptides, and alternative mRNA transcripts are common for all three AE genes. Although AE1 transcripts have been detected in rat heart, AE3 transcripts seem to be the most abundantly expressed in heart (235). In fact, a cardiac variant of the AE3 Cl^-/HCO_3^- exchanger was detected in a rat heart library (257). The predicted cardiac AE3 polypeptide was 1,030 amino acids in length, compared with the 1,227-amino acid AE3 variant expressed in brain, and the cardiac protein contained a unique NH_2-terminal sequence of 73 amino acids that replaced the first 270 amino acids of the brain form. In a subsequent study, the cardiac-specific variant of the AE3 Cl^-/HCO_3^- exchanger (3.6 kb) and a longer 4.4-kb AE3 transcript were found in mouse and human hearts (256). A functional link between AE3 transcripts and Cl^-/HCO_3^- exchange was provided by the finding that similar cardiac AE3 isoforms, cloned and sequenced from a human heart library, gave rise to enhanced $^{36}Cl^-$ uptake when expressed in *Xenopus* oocytes (501). Antiserum against a 80-kDa truncated form of AE1 (nAE1) and antibodies against a 120-kDa translation product of AE3 were shown to significantly inhibit SITS-sensitive Cl^-/HCO_3^- exchange when injected into single adult cardiomyocytes (341). A very recent study suggests that the predominant Cl^-/HCO_3^- exchanger in neonatal and adult rat myocytes may be due to expression of the truncated nAE1 (350).

At least 12 members of the Na^+-dependent Cl^- transporter family have been cloned, 9 of which are from vertebrate cells, indicating considerable molecular diversity due to alternative splicing. The three major vertebrate

subgroups of this family are ENCC1, ENCC2, and ENCC3 (electroneutral Na^+-Cl^- cotransporters; see Refs. 152, 213 for review). Members of all three subgroups exhibit a similar membrane topology, with 12 hydrophobic membrane-spanning segments, a large extracellular loop between the seventh and eighth membrane-spanning segments, and long intracellular NH_2 and COOH termini. The overall sequence homology between the different cotransport proteins is ~45–50%, being greatest in the hydrophobic core and COOH terminal and least in the NH_2 terminus. Functional analysis indicates that ENCC1 may be responsible for Na^+-Cl^- cotransport, whereas ENCC2 and ENCC3 may be responsible for Na^+-K^+-$2Cl^-$ cotransport in different vertebrate cells. ENCC1 and ENCC3 are rather ubiquitiously expressed, the later being identified in heart, whereas ENCC2 seems kidney specific. A variety of differences in functional properties between K^+-Cl^- cotransport and members of the Na^+-dependent Cl^- transporter family (242) suggest that this cotransporter may not be a specialized mode of operation of the Na^+-K^+-$2Cl^-$ cotransporter, although they may be structurally related.

Recent studies suggest that another novel exchange cotransporter may also play a role in regulating a^i_{Cl} in cardiac cells. In guinea pig ventricular myocytes in HCO_3^--free solutions, acid loading in low extracellular pH was DIDS insensitive, independent of Na^+, but reversibly inhibited by extracellular Cl^- removal, suggesting the existence of a novel Cl^--dependent acid influx pathway, possibly involving a Cl^-/OH^- exchanger (CHE) or, alternatively, a H^+-Cl^- coinflux carrier (420). The possibility that this novel acid-loading mechanism might actually be due to Cl^-/HCO_3^- exchange in the presence of residual HCO_3^-, rather than CHE, was ruled out in subsequent experiments in which a similar acid-loading mechanism was observed even under CO_2-free conditions, which should eliminate all residual HCO_3^- (246). Future studies should reveal whether or not CHE has a unique molecular stucture or may be a novel member of the AE gene family of anion exchangers.

Because of their sensitivity to small changes in a^i_{Cl}, another important physiological role of these anion cotransporters and exchangers is cell volume regulation. K^+-Cl^- cotransport and Cl^-/HCO_3^- exchange have been known for some time to participate in mediating RVD in swollen cells, and Na^+-Cl^- cotransport, Na^+-K^+-$2Cl^-$ cotransport, and Cl^-/HCO_3^- exchange participate in mediating regulatory volume increases in shrunken cells (38, 239, 355). Furthermore, the regulation of several of these transporters during changes in cell volume seems to be controlled by protein phosphorylation and dephosphorylation (see Refs. 144, 328 for review). For example, the activation of Na^+-K^+-$2Cl^-$ cotransport during cell shrinkage can be mimicked under isotonic conditions by a variety of serine/threonine phosphatase inhibitors, and shrinkage-induced activation of Na^+-K^+-$2Cl^-$ cotransport is associated with direct phosphorylation of the transport protein (273). A recently proposed model suggests that cell shrinkage in red blood cells may promote transporter phosphorylation by stimulation of an unidentified volume-sensitive protein kinase, rather than inhibition of protein phosphatase (272). In contrast, in the case of K^+-Cl^- cotransport, activation during red blood cell swelling appears to be associated with protein dephosphorylation. Thus activation of K^+-Cl^- cotransport during cell swelling is blocked by serine/threonine phosphatase inhibitors and is stimulated under isotonic conditions by inhibition of endogenous kinase activity (203, 242, 331). It has been suggested that activation of K^+-Cl^- cotransport during cell swelling may be due to inhibition of an unknown volume-sensitive protein kinase (202). Protein kinases that have been shown to be altered during acute changes in cell volume include PKC (241) and a novel serine/threonine protein kinase, termed h-sgk (462). Although considerably more work is required to firmly establish the validity of the phosphorylation/dephosphorylation regulation hypothesis, as well as the identity of the kinase(s) involved, it is interesting that many properties associated with the regulation of K^+-Cl^- cotransport during cell volume changes are similar to those recently described for cell volume-induced changes in ClC-3, a molecular candidate for $I_{Cl.vol}$ (91; Fig. 6).

V. PHYSIOLOGICAL AND CLINICAL SIGNIFICANCE OF SARCOLEMMAL CHLORIDE CHANNELS

Estimates of a^i_{Cl} in cardiac muscle place E_{Cl} in the range of −65 to −45 mV under normal physiological conditions (see sect. IV), a membrane potential range that can be either negative or positive to the actual membrane potential during the normal cardiac cycle. Thus membrane Cl^- channels have the unique ability, compared with cation channels, to contribute both inward as well as outward current during the cardiac action potential (44). As illustrated in Figure 8*A*, at membrane potentials negative to E_{Cl}, activation of I_{Cl} would be expected to produce a small inward current that could potentially depolarize the resting membrane potential, whereas during the action potential plateau, activation of I_{Cl} would be expected to produce outward current and accelerate repolarization. The degree to which activation of I_{Cl} depolarizes the resting potential or accelerates action potential repolarization depends critically on the actual value of E_{Cl} and the magnitude of the Cl^- conductance relative to the total membrane conductance. Because under normal physiological conditions the transmembrane Cl^- gradient is asymmetric, the activation of $I_{Cl.CFTR}$ (through PKA, PKC, or purinergic stimulation; see Table 1) as well as

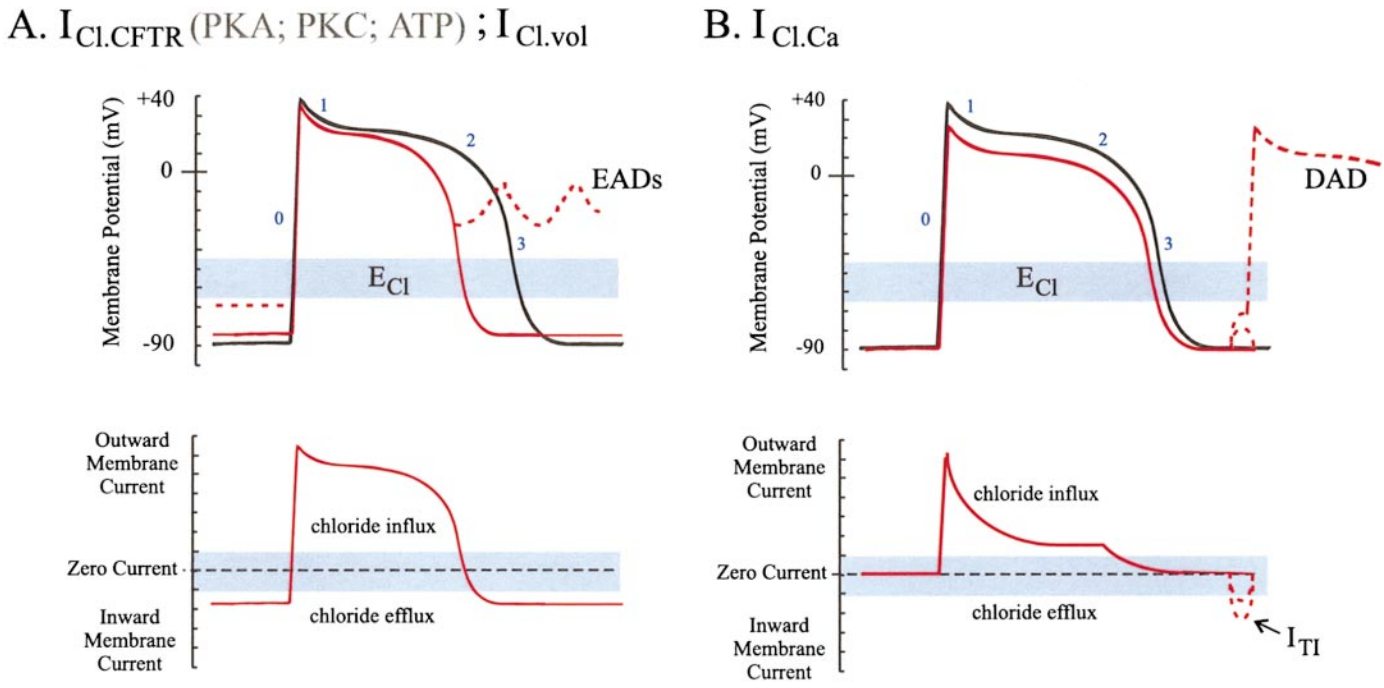


FIG. 8. Modulation of cardiac electrical activity by sarcolemmal Cl^- channels. Changes in action potentials (*top*) and membrane currents (*bottom*) due to activation of CFTR and volume-regulated Cl^- channels (*A*) and Ca^{2+}-activated Cl^- channels (*B*) are depicted. $I_{Cl.CFTR}$ can be activated by stimulation of PKA, PKC, or purinergic receptors; $I_{Cl.vol}$ is activated by cell swelling induced by exposure to hypotonic extracellular solutions or possibly membrane stretch; and $I_{Cl.Ca}$ is activated by elevation of localized $[Ca^{2+}]_i$. Range of estimates for normal physiological values for Cl^- equilibrium potential (E_{Cl}) is indicated in blue in *top panels* in *A* and *B*; range of zero-current values corresponding to E_{Cl} is shown in blue in *bottom panels*. Numbers in blue in *top panels* of *A* and *B* illustrate conventional phases of a prototype ventricular action potential under control conditions (black) and after activation of I_{Cl} (red). In *A*, activation of I_{Cl} induces larger membrane depolarization and induction of early afterdepolarizations (EAD) under conditions where resting K^+ conductance is reduced (dashed red lines); in *B*, activation of $I_{Cl.Ca}$ during $[Ca^{2+}]_i$ overload results in oscillatory transient inward current (I_{TI}) and induction of delayed afterdepolarization (DAD). See text for details.

$I_{Cl.vol}$ will result in an outwardly rectifying I_{Cl} (Fig. 8*A*, *bottom*). This will have a more significant effect at positive membrane potentials to shorten action potential duration compared with smaller depolarizing effects at negative potentials near the resting membrane potential. The ability of I_{Cl} activation to depolarize cardiac cells is also opposed by the presence of a large background K^+ conductance that normally controls the resting membrane potential. In the case of $I_{Cl.PKA}$ (and some other types of I_{Cl} as well), a major physiological role may be to minimize (oppose) the significant action potential prolongation associated with β-adrenergic stimulation of I_{Ca}. This is expected to contribute to action potential shortening during strong adrenergic stimulation and faster heart rates. Myocardial hypokalemia that is known to be arrhythmogenic is expected to reduce background K^+ conductance, and under these conditions activation of I_{Cl} will cause significant membrane depolarization and induce abnormal automaticity leading to the development of early afterdepolarizations (dotted lines in Fig. 8). These predicted effects of $I_{Cl.PKA}$ on action potential duration and automaticity have been verfied experimentally by manipulations of the Cl^- gradient or the use of Cl^- channel antagonists (163, 250, 280, 427, 496). More recently, very similar effects of activation of $I_{Cl.vol}$ on cardiac action potentials have also been observed (87, 449; see sect. II*C9*). This is not too surprising since both $I_{Cl.CFTR}$ and $I_{Cl.vol}$ are relatively time and voltage independent over the physiological range of membrane potentials, although activation of $I_{Cl.vol}$ may be expected to produce more significant action potential shortening due to its stronger outwardly rectifying properties. Activation of either $I_{Cl.CFTR}$ or $I_{Cl.vol}$ may contribute to the development of reentry arrhythmias due to shortening of action potential duration and refractoriness, and possibly due to a slowing of conduction velocity that may result from the small membrane depolarization (174).

As illustrated in Figure 8*B*, the activation of $I_{Cl.Ca}$ will have considerably different effects on cardiac action potentials and resting membrane potential, even though $I_{Cl.Ca}$ is also expected to be outwardly rectifying under physiological conditions (Table 1). This is because $I_{Cl.Ca}$ will exhibit significant kinetic behavior that will largely be determined by the time course of the $[Ca^{2+}]_i$ transient (519). $I_{Cl.Ca}$ will normally have insignificant effects on the diastolic membrane potential, since resting $[Ca^{2+}]_i$ is low. However, a transient outward $I_{Cl.Ca}$ will activate early

during the action potential in response to CICR, and the time course of decline of the $[Ca^{2+}]_i$ transient will determine the extent to which $I_{Cl.Ca}$ contributes to early repolarization during phase 1 (Fig. 8*B*, *bottom*). Under conditions of $[Ca^{2+}]_i$ overload (Fig. 8, dotted lines), spontaneous intracellular Ca^{2+} release can activate arrhythmogenic I_{TI}, partially mediated by $I_{Cl.Ca}$, which can cause delayed afterdepolarizations, an important mechanism of abnormal electrical impulse formation (174).

Most studies that have examined the contribution of I_{Cl} to the cardiac action potential have relied on anion antagonist and substitution experiments. The pharmacological specificity of many of these anion channel antagonists can be problematic, and anion substitution, in addition to altering anion movement through channels, can have other unpredictable side effects on other transport proteins and signaling pathways as well (122, 305). Thus the development of more specific antagonists for each of the different types of cardiac sarolemmal Cl^- channels will significantly contribute to a better understanding of the physiological role of each of these channels in cardiac physiology and help to eliminate possible additional confounding influences due to nonspecific effects of some of the available agents on cation channels. It also is not entirely clear that the agents presently available have always been effective in separating multiple types of I_{Cl} that may overlap during macroscopic current measurements in isolated myocytes. This might be an important factor contributing to some of the recent controversies related to the regulation and pharmacological properties of some types of I_{Cl} in heart.

Another important physiological role of I_{Cl} activation in heart may be cell volume homeostasis. It is well accepted in most cells that activation of $I_{Cl.vol}$ represents one important trigger to initiate RVD (141, 239), and a significant role of $I_{Cl.vol}$ in mediating RVD has been confirmed in cardiac cells (418, 512; see sect. II*C1*), despite the possibility that regulatory volume responses in cardiac myocytes may be compromised somewhat by their low water permeability (417). Other types of I_{Cl} might contribute to RVD as well. Wang et al. (470) have recently shown, following cell swelling by exposure to hypotonic solutions, activation of $I_{Cl.PKA}$ by β-adrenergic activation induced RVD in guinea pig ventricular myocytes. This was explained by activation of $I_{Cl.PKA}$ leading to Cl^- efflux and membrane depolarization, causing increased K^+ efflux and subsequent loss of cellular water. If this explanation is correct, then activation of other types of I_{Cl} might be expected to contribute to RVD in a similar fashion. It would be interesting, for example, to test whether or not activation of $I_{Cl.Ca}$ might also produce RVD. It is not clear from the Wang et al. study (470) why consistent changes in cell volume were not observed in cells exposed to isoproterenol in isotonic solutions, a result similar to reports in rabbit atrial and ventricular myocytes that cAMP and forskolin failed to affect isosmotic cell volume (54, 58).

Because the activation of cardiac Cl^- channels can produce significant effects on action potential duration and automaticity, these channels may have important clinical significance for several myocardial diseases. However, the exact role of Cl^- channels in human cardiac physiology is uncertain, and Cl^- channels have yet to be directly linked to any known human cardiac pathology. Therefore, it should be emphasized that at this time, the potential clinical significance of myocardial Cl^- channels remains speculative and based solely on data obtained from various animal models of human disease.

Chloride channels might be considered novel potential targets for the development of antiarrhythmic agents (111). Specific Cl^- channel antagonists, like existing class III antiarrhythmics which block myocardial K^+ channels, may prolong the refractory period of cardiac muscle and be useful for the prevention of malignant arrhythmias and sudden cardiac death. In relation to this, it has been reported that Cl^- currents may underlie the functional antagonism of class III antiarrhythmics by β-adrenergic agonists (368). It is possible that some exisiting class III antiarrhythmics, which block myocardial K^+ channels, may in addition block Cl^- channels (112). Although the well-known antiarrhythmic and antifibrillatory effects of β-adrenergic blockers are usually attributed to antagonism of I_{Ca} (332), part of their effectiveness might also be related to suppression of $I_{Cl.PKA}$. Because $I_{Cl.PKA}$ is also activated by histamine (166), modulation of these channels, along with I_{Ca}, may also contribute to the occurrence of histamine-induced arrhythmias (486).

Protein kinase A-regulated Cl^- channels could be particularly important when there is a pronounced release of endogenous catecholamines, such as during hypoxia, myocardial swelling, or ischemia. Severe action potential shortening is a characteristic feature of myocardial hypoxia, ischemia, substrate-free anoxia, or complete metabolic inhibition. This shortening of the action potential is usually attributed to activation of K_{ATP} channels (311), although evidence for involvement of $I_{Cl.PKA}$ in the early phase of action potential shortening during hypoxia has also been obtained in perfused rabbit hearts (362) based on anion antagonist and substitution experiments.

There is evidence that Cl^- channels may play a role in myocardial ischemia, congestive heart failure (CHF), and hypertrophy. Chloride ion substitution by NO_3^- has been shown to protect against reperfusion and ischemia-induced arrhythmias in a rat Langendorff model (351). Essentially similar results were reported in an arterially perfused guinea pig ventricular model of no-flow ischemia (429). Both 9-AC and SITS exerted protection against myocardial ischemia-reperfusion damage. These studies, however, were unable to differentiate whether these effects might be mediated by alterations in anion channel

permeability or due to alterations in Cl^-/HCO_3^- exchange, which should also be inhibited by these same experimental interventions and lead to intracellular alkalinization. In a subsequent study (71), evidence that this protection against ischemia-reperfusion arrhythmias might involve anion channels was obtained. It was shown that the protective effects of various anion substitutes could not be explained by changes in intracellular pH and exhibited an order of potency closely matching a known anion permeability sequence for anion channels, which might correspond to $I_{Cl.vol}$. Despite the novelty of these observations, some degree of caution is warranted, since anion substitution can have other effects, and the absolute specificity of compounds such as 9-AC and stilbene derivatives remain suspect. The possible role of $I_{Cl.vol}$ in ischemic preconditioning has not been tested, even though PKC translocation and phosphorylation (which may inhibit activation of $I_{Cl.vol}$, e.g., Fig. 6) has been implicated in this form of cardioprotection (392). New strategies for developing "ischemia-selective" antiarrhythmic agents based on targeting cell swelling-activated or -modulated membrane currents have recently been proposed (488), and targeting of anion channels, particularly $I_{Cl.vol}$, may become one useful approach. However, the development of new, more specific anion-selective experimental agents is required before this approach can reach fruition.

Two recent studies have provided evidence that there may be persistent activation of I_{Cl} in hypertrophied myocytes. Using a rat aortic banding model of hypertropy, Benitah et al. (23) observed an outwardly rectifying Ba^{2+}-resistant current in myocytes from hypertrophied hearts that was not present in myocytes from control hearts. This current was insensitive to monovalent cations, was partially blocked by 9-AC, and was sensitive to variations in Cl^- concentration. Consistent with these findings, 9-AC had no effect on action potentials in control cells but significantly prolonged action potentials in hypertrophied cells, suggesting that persistent activation of I_{Cl} leads to action potential shortening in hypertrophy, which may provide protection against arrhythmias (see sect. II*C9*). In a canine model of tachycardia-induced CHF, persistent activation of a I_{Cl} similar to $I_{Cl.vol}$ has been observed in hypertrophied ventricular myocytes under isosmotic conditions, which is only observed in control myocytes after hypotonic cell swelling (60). Mechanosensitive cation channels are also activated in hypertropied canine myocytes from this same CHF model (59). The persistently activated I_{Cl} was identified as $I_{Cl.vol}$ by its outwardly rectifying properties, sensitivity to block by 9-AC, and inhibition in hypertrophied myocytes by hyperosmotic cell shrinkage. Persistent activation of $I_{Cl.vol}$ in a rabbit aortic regurgitation model of CHF has also been recently observed (56). Persistent activation of $I_{Cl.vol}$ in hypertrophied CHF cells might be mediated by a reduction in the expression of PKC isoforms or a reduction in the particulate PKC fraction that have been documented to occur in some CHF models (294, 359). Such a mechanism would be consistent with the model proposed in Figure 6 suggesting a role of PKC phosphorylation in the regulation of ClC-3 Cl^- channels by changes in cell volume (57, 91). Alternatively, persistent activation of $I_{Cl.vol}$ might involve CHF-induced changes in tyrosine kinase (364, 402; see sect. II*C4*).

Finally, although not yet specifically tested, it is possible that $I_{Cl.PKA}$, $I_{Cl.Ca}$, and $I_{Cl.vol}$ might play a role in the genesis of mechanical- or stretch-induced cardiac arrhythmias (124, 237). Myocardial stretch is known to release catecholamines as well as directly elevate $[Ca^{2+}]_i$ (277, 295, 369). Stretch-activated ion channels have been suggested to contribute to the development of stretch-induced membrane depolarizations and arrhythmias (125, 406), and mathematical membrane models incorporating SAC successfully reproduce many features characteristic of stretch-induced changes in myocardial excitability measured experimentally (352, 363, 503).

VI. CHLORIDE CHANNELS IN INTRACELLULAR MEMBRANES

A. Sarcoplasmic Reticular Membranes

Calcium-induced Ca^{2+} release in cardiac myocytes is well known to be mediated by ryanodine receptors located in the SR. Potassium channels and Cl^- channels are also expressed in the SR membrane, whose functions include maintenance of electroneutrality of the overall transport process, SR pH and osmoregulation (172, 416, 421), and possibly ion exchange (310). The incorporation of SR vesicles into planar lipid bilayers provided early evidence of Cl^- channel expression in cardiac SR. A 55-pS (in 260 mM Cl^-) anion-selective channel was described for canine ventricular SR, which was voltage dependent and inhibited by DIDS (360). A similar 116-pS (in 500 mM Cl^-) channel was described in vesicles isolated from porcine SR (218). The channel appeared to spontaneously inactivate with time in the bilayer, a process that could be reversed by exposure of the *cis*-side to PKA catalytic subunit and MgATP. This channel was nearly completely blocked by the stilbene derivative DNDS. A subsequent study (216) showed this PKA-regulated SR Cl^- channel to also be inhibited by Ca^{2+}/calmodulin, suggesting an important role in SR function during contraction and relaxation. Voltage-dependent reactivation and deactivation of a similar SR Cl^- channel in vesicles from sheep SR may be due to association and dissociation of a regulatory subunit, possibly phospholamban (76). Phospholamban also appears to regulate the activity of a Cl^- channel derived from human atrial SR vesicles (75).

B. Nuclear Membranes

One mechanism by which stimuli that alter gene expression may transmit their signals into the nucleus may involve alterations in the permeability of the nuclear membrane. A variety of different channels including K^+, Cl^-, nonselective cationic channels, and IP_3-gated Ca^{2+} channels gated have been identified in the nuclear envelopes of a variety of cell types (33, 274, 285, 408; see Refs. 34, 245, 409 for review). Like in SR membranes, Cl^- channels in the nuclear envelope may represent a counter-charge transport pathway that functions to maintain overall electroneutrality (422). Bilayer reconstitution experiments using nuclear membrane fractions isolated from sheep myocardium have revealed two distinct types of Cl^--selective channels (361). These include a large-conductance (150–180 pS) Cl^- channel, modulated by retinoic acid, and a smaller conductance (30 pS) Cl^- channel, modulated by ATP. These channels appear to be similar to the large- and small-conductance Cl^- channels previously detected using patch-clamp techniques on the nuclear outer membrane of liver cells (422).

C. Mitochondrial Membranes

A number of anion channels have been identified in the outer and inner membranes of mitchondria (see Refs. 14, 119, 398 for reviews). A VDAC in the outer mitchondrial membrane (mitochondrial porin) is a small 283-amino acid protein found in most eukaryotic cells. Voltage-dependent anion channels form large-conductance (4–5 nS) channels that are approximately twice as selective for Cl^- over K^+ and are also thought to represent a large aqueous conduction pathway. A VDAC is believed to function as a pathway for the movement of adenine nucleotides and other metabolites through the mitochondrial membrane (358). Two primary types of anion-selective channels have been identified in cardiac mitoplasts (inner mitochondrial membrane vesicles) from rat, mouse, and sheep hearts (226, 399, 515). These include a 45- to 50-pS channel that is regulated by pH and Mg^{2+} and is thought to be important in mitochondrial volume regulation and a 100- to 107-pS anion channel regulated by nucleotides. The larger conductance channel exhibited multisubstates, and both channels were insensitive to SITS. The smaller conductance channel exhibited substate behavior consistent with a multibarrelled channel containing four functionally coupled pores. The molecular basis of these channels and their relationship to the DIDS- and SITS-sensitive IMAC, characterized in flux studies on intact mitochondria (20), is unknown.

VII. CONCLUSIONS AND FUTURE DIRECTIONS

In the 10 years since cardiac Cl^- channels were "rediscovered," a great deal of progress has been made in the functional and molecular identification of these channels. Although early functional studies provided evidence for the existence of at least six different sarcolemmal Cl^- channels in heart (2, 97), at this time it appears that this number can be reduced to four primary functional sarcolemmal channel subtypes: $I_{Cl.PKA}$, $I_{Cl.vol}$, $I_{Cl.Ca}$, and $I_{Cl.ir}$ (Table 1). Chloride conductances present under basal conditions, and those activated by stimulation of PKC or membrane purinergic receptors appear to be mediated by one of these three major Cl^- channel subtypes. $I_{Cl.PKA}$ in heart is encoded, in most species, by an isoform of the epithelial CFTR Cl^- channel, and $I_{Cl.vol}$ and $I_{Cl.ir}$ may be encoded by members of the ClC Cl^- channel gene family, ClC-3 and ClC-2, respectively. Although a type of $I_{Cl.Ca}$ may be encoded by CLCA1 in some mammalian cells, it remains to be determined whether or not a member of the CLCA gene family is responsible for $I_{Cl.Ca}$ in heart. Indications that at least six members of the ClC gene family may be expressed in heart, along with the possibility that these may coassemble to form heteromultimers, provide a rich source of potential molecular candidates that may encode existing or novel Cl^- channel subtypes in sarcolemmal and intracellular membranes.

Despite this recent progress, however, the field lags far behind that of cation channels, and several crucial developments need to be made to ensure continued advancement. Perhaps of primary importance is the identification of a common anion pore structure. The identification of a conserved consensus sequence for an anion pore would allow investigators to search databases of sequences for putative anion channels as well as provide the basis for probes to screen cardiac libraries. The ClC channels are providing credible clues in this area, but the data that are being generated are demonstrating that elucidation of this problem will be a formidable one. Fahlke et al. (110) have located a core structural element (P1 region) of the ClC channel pore that spans an accessibility barrier between the internal and external milieu and contains an evolutionarily conserved sequence motif, GKxG-PxxH. However, several other regions in quite distant domains of the ClC primary structure also appear to contribute to anion permeation. Mutating amino acids in the NH_2 terminus or transmembrane spanning domains (269) and the COOH terminus (343) also alter rectification and ion permeation properties of ClC-0 and ClC-1. Overall, the domains that contribute to the anion permeation pathway for ClC channels may form a quite complex structure, in contrast to the symmetrical and comprehendible pore structure for cation channels (see Ref. 86). In addition, the multimeric nature of ClC structure has not been conclusively determined (see sect. II*F*). Therefore, a

symmetrical nature to the anion pore has not been established, and the amino acids that participate in anion permeation may depend on the subunit of which they are a part.

The primary and secondary structure for the anion permeation pathway for other, non-ClC channels (e.g., CFTR) may be very different from the ClC family of channels. Therefore, cardiac Cl^- channels in sarcolemmal as well as internal membranes that, as yet, do not have a known molecular counterpart may fall into either of these structures. In addition, there may be an entirely novel anion-selective structure that has yet to be identified and may underlie some of these "orphan" Cl^- channels.

The importance of determining the molecular species responsible for cardiac Cl^- transport proteins cannot be overemphasized. The difficulty in determining the functional, physiological, and pathophysiological importance of these carriers and channels is based on the lack of specific pharmacological tools. With the identification of well-defined molecular structures, new drugs can be designed to target particular regions (particularly the anion conduction pathway and outer pore vestibule regions of channels) to improve potency and specificity of available agents. Such developments are necessary to elucidate the physiological and clinical significance of these often neglected transport proteins.

An important caveat to consider in future molecular biological studies of cardiac tissues and cells are anion channels and carriers in intracellular membranes. Because transcripts encoding these will also be represented in any cardiac RNA preparation or library, it will be essential to determine whether the resulting cDNA encodes a plasma membrane or internal membrane channel or carrier protein.

Finally, insights into the molecular genetics of cardiac Cl^- transport proteins are an essential first step toward identification of natural mutations in these genes that may significantly affect cardiac function. Interestingly, human Cl^- channel mutations are widespread and responsible for a variety of diseases (1, 206, 249, 477). Molecular genetic studies have recently provided new insights into the role of cation channels and other proteins and signaling molecules in inherited cardiac arrhythmias and cardiomyopathies (221). The application of similar approaches to determine the role of anion channels and carriers in inherited cardiovascular diseases could be quite revealing.

We acknowledge the following individuals for invaluable discussions: Clive Baumgarten, David Dawson, Denis Escande, David Gadsby, Robert Harvey, Paul Levesque, Randall Moorman, Eric Rousseau, Jamie Vandenburg, and Kevin Strange. Additional thanks to James Kenyon for comments on the manuscript.

The authors' work was supported by National Heart, Lung, and Blood Institute Grants HL-52803 and HL-49254.

Present addresses: M. L. Collier, Dept. of Animal Biology, Univ. of Pennsylvania College of Veterinary Medicine, 3800 Spruce St., Philadelphia, PA 19104-6046; and J. Yamazaki, Dept. of Pharmacology, Fukuoka Dental College, 2–15–1 Tamura, Swara-ku, Fukuoka 814–0139, Japan.

Address for reprint requests and other correspondence: J. R. Hume, Dept. of Physiology & Cell Biology/351, Univ. of Nevada School of Medicine, Reno, NV 89557-0046 (E-mail: joeh@med.unr.edu).

REFERENCES

1. ACKERMAN, M., AND D. E. CLAPHAM. Mechanisms of disease: ion channels-basic science and clinical disease. *N. Engl. J. Med.* 336: 1575–1586, 1997.
2. ACKERMAN, M. J., AND D. E. CLAPHAM. Cardiac chloride channels. *Trends Cardiovasc. Med.* 3: 23–28, 1993.
3. ACKERMAN, M. J., K. D. WICKMAN, AND D. E. CLAPHAM. Hypotonicity activates a native chloride current in *Xenopus* oocytes. *J. Gen. Physiol.* 103: 153–179, 1994.
4. ADLER, D. A., E. I. RUGARLI, P. A. LINGENFELTER, K. TSUCHIYA, D. POSLINSKI, H. D. LIGGITT, V. M. CHAPMAN, R. W. ELLIOTT, A. BALLABIO, AND C. M. DISTECHE. Evidence of evolutionary up-regulation of the single active X chromosome in mammals based on Clc4 expression levels in *Mus spretus* and *Mus musculus*. *Proc. Natl. Acad. Sci. USA* 94: 9244–9248, 1997.
5. AGUILAR-BRYAN, L., C. G. NICHOLS, S. W. WECHSLER, J. P. CLEMENT, A. E. BOYD, G. GONZALEZ, H. HERRERA-SOSA, K. NGUY, J. BRYAN, AND D. A. NELSON. Cloning of the beta cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. *Science* 268: 423–426, 1995.
6. AKIYAMA, T., AND H. A. FOZZARD. Influence of potassium ions and osmolality on the resting membrane potential of rabbit ventricular papillary muscle with estimation of the activity and the activity coefficient of internal potassium. *Circ. Res.* 37: 621–629, 1975.
7. ALPER, S. L. The band 3-related anion exchanger (AE) gene family. *Annu. Rev. Physiol.* 53: 549–564, 1991.
8. ANDERSON, M. P., H. A. BERGER, D. P. RICH, R. J. GREGORY, A. E. SMITH, AND M. J. WELSH. Nucleoside triphosphates are required to open the CFTR chloride channel. *Cell* 67: 775–784, 1991.
9. ANDERSON, M. P., R. J. GREGORY, S. THOMPSON, D. W. SOUZA, S. PAUL, R. C. MULLIGAN, A. E. SMITH, AND M. J. WELSH. Demonstration that CFTR is a chloride channel by alteration of its anion selectivity. *Science* 253: 202–205, 1991.
10. ANDERSON, M. P., D. P. RICH, R. J. GREGORY, A. E. SMITH, AND M. J. WELSH. Generation of cAMP-activated chloride currents by expression of CFTR. *Science* 251: 679–682, 1991.
11. ANDERSON, M. P., AND M. J. WELSH. Regulation by ATP and ADP of CFTR chloride channels that contain mutant nucleotide-binding domains. *Science* 257: 1701–1704, 1992.
12. ARAI, A., I. KODAMA, AND J. TOYAMA. Roles of Cl^- channels and Ca^{2+} mobilization in stretch-induced increase of SA node pacemaker activity. *Am. J. Physiol.* 270 (*Heart Circ. Physiol.* 39): H1726—H1735, 1996.
13. BAHINSKI, A., A. C. NAIRN, P. GREENGARD, AND D. C. GADSBY. Chloride conductance regulated by cyclic AMP-dependent protein kinase in cardiac myocytes. *Nature* 340: 718–721, 1989.
14. BALLARIN, C., A. BERTOLI, G. WOJCIK, AND M. C. SORGATO. Mitochondrial inner membrane channels in yeast and mammals. *Soc. Gen. Physiol. Ser.* 51: 155–171, 1996.
15. BARNARD, E. A., P. SKOLNICK, R. W. OLSEN, H. MOHLER, W. SIEGHART, G. BIGGIO, C. BRAESTRUP, A. N. BATESON, AND S. Z. LANGER. International Union of Pharmacology. XV. Subtypes of gamma-aminobutyric $acid_A$ receptors: classification on the basis of subunit structure and receptor function. *Pharmacol. Rev.* 50: 291–313, 1998.
16. BARRY, D. M., AND J. M. NERBONNE. Myocardial potassium channels: electrophysiological and molecular diversity. *Annu. Rev. Physiol.* 58: 363–394, 1996.
17. BAUKROWITZ, T., T. C. HWANG, A. C. NAIRN, AND D. C. GADSBY.

Coupling of CFTR Cl^- channel gating to an ATP hydrolysis cycle. *Neuron* 12: 473–482, 1994.
18. BAUMGARTEN, C. M., AND S. W. N. DUNCAN. Regulation of Cl^- activity in ventricular muscle: Cl^-/HCO_3^- excange and Na^+-dependent Cl^- cotransport. In: *Heart Function and Metabolism*, edited by N. S. Dhalla. Boston, MA: Nijhoff, 1987, p. 117–131.
19. BAUMGARTEN, C. M., AND H. A. FOZZARD. Intracellular chloride activity in mammalian ventricular muscle. *Am. J. Physiol.* 241 (*Cell Physiol.* 10): C121—C129, 1981.
20. BEAVIS, A. D. Properties of the inner membrane anion channel in intact mitochondria. *J. Bioenerg. Biomembr.* 24: 77–90, 1992.
21. BECK, C. L., C. FAHLKE, AND A. L. J. GEORGE. Molecular basis for decreased muscle chloride conductance in the myotonic goat. *Proc. Natl. Acad. Sci. USA* 93: 11248–11252, 1996.
22. BECQ, F., T. J. JENSEN, X. B. CHANG, A. SAVOIA, J. M. ROMMENS, L. C. TSUI, M. BUCHWALD, J. R. RIORDAN, AND J. W. HANRAHAN. Phosphatase inhibitors activate normal and defective CFTR chloride channels. *Proc. Natl. Acad. Sci. USA* 91: 9160–9164, 1994.
23. BENITAH, J. P., A. M. GOMEZ, C. DELGADO, P. LORENTE, AND W. J. LEDERER. A chloride current component induced by hypertrophy in rat ventricular myocytes. *Am. J. Physiol.* 272 (*Heart Circ. Physiol.* 41): H2500—H2506, 1997.
24. BERGER, H. A., S. M. TRAVIS, AND M. J. WELSH. Regulation of the cystic fibrosis transmembrane conductance regulator Cl^- channel by specific protein kinases and protein phosphatases. *J. Biol. Chem.* 268: 2037–2047, 1993.
25. BERUL, C. I., T. SWEETEN, V. L. VETTER, AND M. MORAD. Lack of cystic fibrosis transmembrane regulator-type chloride current in pediatric human atrial myocytes. *Life Sci.* 60: 189–197, 1997.
26. BESCOND, J., P. BOIS, J. PETIT-JACQUES, AND J. LENFANT. Characterization of an angiotensin-II-activated chloride current in rabbit sino-atrial cells. *J. Membr. Biol.* 140: 153–161, 1994.
27. BOND, T. D., M. A. VALVERDE, AND C. F. HIGGINS. Protein kinase C phosphorylation disengages human and mouse-1a P-glycoproteins from influencing the rate of activation of swelling-activated chloride currents. *J. Physiol. (Lond.)* 508: 333–340, 1998.
28. BORTNER, C. D., AND J. A. CIDLOWSKI. Absence of volume regulatory mechanisms contributes to the rapid activation of apoptosis in thymocytes. *Am. J. Physiol.* 261 (*Cell Physiol.* 30): C950—C961, 1996.
29. BOTON, R., D. SINGER, AND N. DASCAL. Inactivation of calcium-activated chloride conductance in *Xenopus* oocytes: roles of calcium and protein kinase C. *Pflügers Arch.* 416: 1–6, 1990.
30. BRANDT, S., AND T. J. JENTSCH. ClC-6 and ClC-7 are two novel broadly expressed members of the CLC chloride channel family. *FEBS Lett.* 377: 15–20, 1995.
31. BREMER, S., T. HOOF, M. WILKE, R. BUSCHE, B. SCHOLTE, J. R. RIORDAN, G. MAASS, AND B. TUMMLER. Quantitative expression patterns of multidrug-resistance P-glycoprotein (*MDR1*) and differentially spliced cystic-fibrosis transmembrane-conductance regulator mRNA transcripts in human epithelia. *Eur. J. Biochem.* 206: 137–149, 1992.
32. BULTMANN, R., AND K. STARKE. Blockade by 4,4′-diisothiocyanatostilbene-2,2′-disulphonate (DIDS) of P_{2X}-purinoceptors in rat vas deferens. *Br. J. Pharmacol.* 112: 690–694, 1994.
33. BUSTAMANTE, J. O., J. A. HANOVER, AND A. LIEPINS. The ion channel behavior of the nuclear pore complex. *J. Membr. Biol.* 146: 239–251, 1995.
34. BUSTAMANTE, J. O., A. LIEPINS, AND J. A. HANOVER. Nuclear pore complex ion channels (review). *Mol. Membr. Biol.* 11: 141–150, 1994.
35. CABANTCHIK, Z. I., AND R. GREGER. Chemical probes for anion transporters of mammalian cell membranes. *Am. J. Physiol.* 262 (*Cell Physiol.* 31): C803—C827, 1992.
36. CAHALAN, M., AND R. S. LEWIS. Role of potassium and chloride channels in volume regulation by T lymphocytes. In: *Cell Physiology of Blood*, edited by R. Gunn and J. Parker. New York: Rockefeller Univ. Press, 1988, p. 281–301.
37. CAILLE, J. P., E. RUIZ-CERETTI, AND O. F. SCHANNE. Intracellular chloride activity in rabbit papillary muscle: effect of ouabain. *Am. J. Physiol.* 240 (*Cell Physiol.* 9): C183—C188, 1981.
38. CALA, P. M. Principles of cell volume regulation. Ion flux pathways and the roles of anions. In: *Chloride Channels and Carriers in Nerve, Muscle and Glial Cells*, edited by F. J. Alvarez-Leefmans and J. M. Russell. New York: Plenum, 1989, p. 67–83.
39. CALLEWAERT, G. Excitation-contraction coupling in mammalian cardiac cells. *Cardiovasc. Res.* 26: 923–932, 1992.
40. CANTIELLO, H. F. Role of the actin cytoskeleton in the regulation of the cystic fibrosis transmembrane conductance regulator. *Exp. Physiol.* 81: 505–514, 1996.
41. CANTIELLO, H. F., A. G. PRAT, I. L. REISIN, L. B. ERCOLE, E. H. ABRAHAM, J. F. AMARA, R. J. GREGORY, AND D. A. AUSIELLO. External ATP and its analogs activate the cystic fibrosis transmembrane conductance regulator by a cyclic AMP-independent mechanism. *J. Biol. Chem.* 269: 11224–11232, 1994.
42. CAREW, M. A., AND P. THORN. Identification of ClC-2-like chloride currents in pig pancreatic acinar cells. *Pflügers Arch.* 433: 84–90, 1996.
43. CARMELIET, E. Chloride ions and the membrane potential of Purkinje fibers. *J. Physiol. (Lond.)* 156: 375–388, 1961.
44. CARMELIET, E. Mechanisms and control of repolarization. *Eur. Heart J.* 14: 3–13, 1993.
45. CARSON, M. R., S. M. TRAVIS, AND M. J. WELSH. The two nucleotide-binding domains of cystic fibrosis transmembrane conductance regulator (CFTR) have distinct functions in controlling channel activity. *J. Biol. Chem.* 270: 1711–1717, 1995.
46. CAYRE, A., N. MOINS, F. FINAT-DUCLOS, J. MAUBLANT, E. ALBUISSON, AND P. VERRELLE. In vitro detection of the MDR phenotype in rat myocardium: use of PCR, [^{3}H]daunomycin and MDR reversing agents. *Anticancer Drugs* 7: 833–837, 1996.
47. CHEN, Z., L. R. JONES, J. J. O'BRIAN, J. R. MOORMAN, AND S. E. CALA. Structural domains in phospholemman: a possible role for the carboxyl terminus in channel inactivation. *Circ. Res.* 82: 367–374, 1998.
48. CHENG, S. H., D. P. RICH, J. MARSHALL, R. J. GREGORY, M. J. WELSH, AND A. E. SMITH. Phosphorylation of the R domain by cAMP-dependent protein kinase regulates the CFTR chloride channel. *Cell* 66: 1027–1036, 1991.
49. CHEUNG, M., AND M. H. AKABAS. Identification of cystic fibrosis transmembrane conductance regulator channel-lining residues in and flanking the M6 membrane-spanning segment. *Biophys. J.* 70: 2688–2695, 1996.
50. CHEUNG, M., AND M. H. AKABAS. Locating the anion-selectivity filter of the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel. *J. Gen. Physiol.* 109: 289–299, 1997.
51. CHIANG, C.-E., S.-A. CHEN, M.-S. CHANG, C.-I. LIN, AND H.-N. LUK. Genistein directly inhibits L-type calcium currents but potentiates cAMP-dependent chloride currents in cardiomyocytes. *Biochem. Biophys. Res. Commun.* 223: 598–603, 1996.
52. CLAPHAM, D. E. The list of potential volume-sensitive chloride currents continues to swell. *J. Gen. Physiol.* 111: 623–624, 1998.
53. CLARK, S., S. E. JORDT, T. J. JENTSCH, AND A. MATHIE. Characterization of the hyperpolarization-activated chloride current in dissociated rat sympathetic neurons. *J. Physiol. (Lond.)* 506: 665–678, 1998.
54. CLEMO, H. F., AND C. M. BAUMGARTEN. cGMP and atrial natriuretic factor regulate cell volume of rabbit atrial myocytes. *Circ. Res.* 77: 741–749, 1995.
55. CLEMO, H. F., AND C. M. BAUMGARTEN. Swelling-activated Gd^{3+}-sensitive cation current and cell volume regulation in rabbit ventricular myocytes. *J. Gen. Physiol.* 110: 297–312, 1997.
56. CLEMO, H. F., AND C. M. BAUMGARTEN. Protein kinase C activation blocks $I_{Cl(swell)}$ and causes myocyte swelling in a rabbit congestive heart failure model. *Circulation* 98, *Suppl.* 1: I-695, 1998.
57. CLEMO, H. F., J. S. DANETZ, AND C. M. BAUMGARTEN. Does ClC-3 modulate cardiac cell volume? (Abstract). *Biophys. J.* 76: A203, 1999.
58. CLEMO, H. F., J. J. FEHER, AND C. M. BAUMGARTEN. Modulation of rabbit ventricular cell volume and $Na^+/K^+/2Cl^-$ cotransport by cGMP and atrial natriuretic factor. *J. Gen. Physiol.* 100: 89–114, 1992.
59. CLEMO, H. F., B. S. STAMBLER, AND C. M. BAUMGARTEN. Persistent activation of a swelling-activated cation current in ventricular myocytes from dogs with tachycardia-induced congestive heart failure. *Circ. Res.* 83: 147–157, 1998.

60. CLEMO, H. F., B. S. STAMBLER, AND C. M. BAUMGARTEN. Swelling-activated chloride current is persistently activated in ventricular myocytes from dogs with tachycardia-induced congestive heart failure. *Circ. Res.* 84: 157–165, 1999.
61. COCA-PRADOS, M., J. ANGUITA, M. L. CHALFANT, AND M. M. CIVAN. PKC-sensitive Cl^- channels associated with ciliary epithelial homologue of pICln. *Am. J. Physiol.* 268 (*Cell Physiol.* 37): C572—C579, 1995.
62. COCA-PRADOS, M., J. SANCHEZ-TORRES, K. PETERSON-YANTORNO, AND M. M. CIVAN. Association of ClC-3 channel with Cl^- transport by human nonpigmented ciliary epithelial cells. *J. Membr. Biol.* 150: 197–208, 1996.
63. COLLIER, M. L., AND J. R. HUME. Unitary chloride channels activated by protein kinase C in guinea pig ventricular myocytes. *Circ. Res.* 76: 317–324, 1995.
64. COLLIER, M. L., P. C. LEVESQUE, J. L. KENYON, AND J. R. HUME. Unitary Cl^- channels activated by cytoplasmic Ca^{2+} in canine ventricular myocytes. *Circ. Res.* 78: 936–944, 1996.
65. CONTE-CAMERINO, D., M. MAMBRINI, A. DELUCA, D. TRICARICO, S. H. BRYANT, V. TORTORELLA, AND G. BETTONI. Enantiomers of clofibric acid analogs have opposite actions on rat skeletal muscle chloride channels. *Pflügers Arch.* 413: 105–107, 1988.
66. CORABOEUF, E., AND E. CARMELIET. Existence of two transient outward currents in sheep cardiac Purkinje fibers. *Pflügers Arch.* 392: 352–359, 1982.
67. CORABOEUF, E., AND J. NARGEOT. Electrophysiology of human cardiac cells. *Cardiovasc. Res.* 27: 1713–1725, 1993.
68. COULOMBE, A., AND E. CORABOEUF. Large-conductance chloride channels of newborn rat cardiac myocytes are activated by hypotonic media. *Pflügers Arch.* 422: 143–150, 1992.
69. CRUMB, W. J. J., J. D. PIGOTT, AND C. W. CLARKSON. Comparison of I_{to} in young and adult human atrial myocytes: evidence for developmental changes. *Am. J. Physiol.* 268 (*Heart Circ. Physiol.* 37): H1335—H1342, 1995.
70. CUNNINGHAM, S. A., M. S. AWAYDA, J. K. BUBIEN, I. I. ISMAILOV, M. P. ARRATE, B. K. BERDIEV, D. J. BENOS, AND C. M. FULLER. Cloning of an epithelial chloride channel from bovine trachea. *J. Biol. Chem.* 270: 31016–31026, 1995.
71. CURTIS, M. J., P. B. GARLICK, AND P. D. RIDLEY. Anion manipulation, a novel antiarrhythmic approach: mechanism of action. *J. Mol. Cell. Cardiol.* 25: 417–436, 1993.
72. DE, G. C., J. SEHRER, F. VIANA, A. K. VAN, J. EGGERMONT, L. MERTENS, L. RAEYMAEKERS, G. DROOGMANS, AND B. NILIUS. Volume-activated chloride currents are not correlated with P-glycoprotein expression. *Biochem. J.* 307: 713–718, 1995.
73. DEAL, K. K., S. K. ENGLAND, AND M. M. TAMKUN. Molecular physiology of cardiac potassium channels. *Physiol. Rev.* 76: 49–67, 1996.
74. DECHECCHI, M. C., R. ROLFINI, A. TAMANINI, C. GAMBERI, G. BERTON, AND G. CABRINI. Effect of modulation of protein kinase C on the cAMP-dependent chloride conductance in T84 cells. *FEBS Lett.* 311: 25–28, 1992.
75. DECROUY, A., M. JUTEAU, S. PROTEAU, J. TEIJIERA, AND E. ROUSSEAU. Biochemical regulation of sarcoplasmic reticulum Cl^- channel from human atrial myocytes: involvement of phospholamban. *J. Mol. Cell. Cardiol.* 28: 767–780, 1996.
76. DECROUY, A., M. JUTEAU, AND E. ROUSSEAU. Examination of the role of phosphorylation and phospholamban in the regulation of the cardiac sarcoplasmic reticulum Cl^- channel. *J. Membr. Biol.* 146: 315–326, 1995.
77. DELANEY, S. J., D. P. RICH, S. A. THOMSON, M. R. HARGRAVE, P. K. LOVELOCK, M. J. WELSH, AND B. J. WAINWRIGHT. Cystic fibrosis transmembrane conductance regulator splice variants are not conserved and fail to produce chloride channels. *Nature Genet.* 4: 426–431, 1993.
78. DEVIDAS, S., AND W. B. GUGGINO. CFTR: domains, structure, and function. *J. Bioenerg. Biomembr.* 29: 443–451, 1997.
79. DIAMOND, G., T. F. SCANLIN, M. A. ZASLOFF, AND C. L. BEVINS. A cross-species analysis of the cystic fibrosis transmembrane conductance regulator. Potential functional domains and regulatory sites. *J. Biol. Chem.* 266: 22761–22769, 1991.
80. DICK, G. M., K. A. KUENZLI, B. HOROWITZ, J. R. HUME, AND K. M. SANDERS. Functional and molecular identification of a chloride conductance in canine colonic smooth muscle. *Am. J. Physiol.* 275 (*Cell Physiol.* 44): C940—C950, 1998.
81. DIFRANCESCO, D. Cardiac pacemaker: 15 years of "new" interpretation. *Acta Cardiol.* 50: 413–427, 1995.
82. DIFRANCESCO, D., AND P. TORTORA. Direct activation of cardiac pacemaker channels by intracellular cyclic AMP. *Nature* 351: 145–147, 1991.
83. DOROSHENKO, P., AND E. NEHER. Volume-sensitive chloride conductance in bovine chromaffin cell membrane. *J. Physiol. (Lond.)* 449: 197–218, 1992.
84. DOUSMANIS, A. G. G. Anion permeability sequence of the open cardiac CFTR Cl channel (Abstract). *Biophys. J.* 66: A421, 1994.
85. DOVE, S. K., F. T. COOKE, M. R. DOUGLAS, L. G. SAYERS, P. J. PARKER, AND R. H. MICHELL. Osmotic stress activates phosphatidylinositol-3,5-bisphosphate synthesis. *Nature* 390: 187–192, 1997.
86. DOYLE, D. A., J. M. CABRAL, R. A. PFUETZNER, A. L. KUO, J. M. GULBIS, S. L. COHEN, B. T. CHAIT, AND R. MACKINNON. The structure of the potassium channel: molecular basis of K^+ conduction and selectivity. *Science* 280: 69–77, 1998.
87. DU, X. Y., AND S. SOROTA. Cardiac swelling-induced chloride current depolarizes canine atrial myocytes. *Am. J. Physiol.* 272 (*Heart Circ. Physiol.* 41): H1904—H1916, 1997.
88. DU, X. Y., AND S. SOROTA. Modulation of dog atrial swelling-induced chloride current by cAMP: protein kinase A-dependent and -independent pathways. *J. Physiol. (Lond.)* 500: 111–122, 1997.
89. DU, X. Y., AND S. SOROTA. Protein kinase C stimulates swelling-induced chloride current in canine atrial cells. *Pflügers Arch.* 437: 227–234, 1999.
90. DUAN, D., F. BRITTON, L. YE, B. HOROWITZ, AND J. R. HUME. A novel anionic inward rectifier in cardiac myocytes encoded by ClC-2 (Abstract). *Biophys. J.* 76: A147, 1999.
91. DUAN, D., S. COWLEY, B. HOROWITZ, AND J. R. HUME. A serine residue in ClC-3 links phosphorylation-dephosphorylation to chloride channel regulation by cell volume. *J. Gen. Physiol.* 113: 57–70, 1999.
92. DUAN, D., B. FERMINI, AND S. NATTEL. Alpha-adrenergic control of volume-regulated Cl^- currents in rabbit atrial myocytes. Characterization of a novel ionic regulatory mechanism. *Circ. Res.* 77: 379–393, 1995.
93. DUAN, D., J. R. HUME, AND S. NATTEL. Evidence that outwardly rectifying Cl^- channels underlie volume-regulated Cl^- currents in heart. *Circ. Res.* 80: 103–113, 1997.
94. DUAN, D., AND S. NATTEL. Properties of single outwardly rectifying Cl^- channels in heart. *Circ. Res.* 75: 789–795, 1994.
95. DUAN, D., C. WINTER, S. COWLEY, J. R. HUME, AND B. HOROWITZ. Molecular identification of a volume-regulated chloride channel. *Nature* 390: 417–421, 1997.
96. DUAN, D., L. YE, F. BRITTON, L. MILLER, J. YAMAZAKI, B. HOROWITZ, AND J. R. HUME. Purinergic-coupled Cl^- channels in mouse heart: a novel, alternative pathway for CFTR regulation. *J. Physiol. (Lond.)* 521: 43–56, 1999.
97. DUAN, D. Y., B. FERMINI, AND S. NATTEL. Sustained outward current observed after $I_{(to1)}$ inactivation in rabbit atrial myocytes is a novel Cl^- current. *Am. J. Physiol.* 263 (*Heart Circ. Physiol.* 32): H1967—H1971, 1992.
98. DUKES, I. D., L. CLEEMANN, AND M. MORAD. Tedisamil blocks the transient and delayed rectifier K^+ currents in mammalian cardiac and glial cells. *J. Pharmacol. Exp. Ther.* 254: 560–569, 1990.
99. EGAN, T. M., D. NOBLE, S. J. NOBLE, T. POWELL, AND V. W. TWIST. An isoprenaline activated sodium-dependent inward current in ventricular myocytes. *Nature* 328: 634–637, 1987.
100. EGAN, T. M., D. NOBLE, S. J. NOBLE, T. POWELL, V. W. TWIST, AND K. YAMAOKA. On the mechanism of isoprenaline- and forskolin-induced depolarization of single guinea-pig ventricular myocytes. *J. Physiol. (Lond.)* 400: 299–320, 1988.
101. EHARA, T., AND K. ISHIHARA. Anion channels activated by adrenaline in cardiac myocytes. *Nature* 347: 284–286, 1990.
102. EHARA, T., AND H. MATSUURA. Single-channel study of the cyclic AMP-regulated chloride current in guinea-pig ventricular myocytes. *J. Physiol. (Lond.)* 464: 307–320, 1993.
103. EHARA, T., A. NOMA, AND K. ONO. Calcium-activated non-selective

cation channel in ventricular cells isolated from adult guinea-pig hearts. *J. Physiol. (Lond.)* 403: 117–133, 1988.
104. EHRING, G. R., Y. V. OSIPCHUK, AND M. D. CAHALAN. Swelling-activated chloride channels in multidrug-sensitive and -resistant cells. *J. Gen. Physiol.* 104: 1129–1161, 1994.
105. EMMA, F., S. BRETON, R. MORRISON, S. WRIGHT, AND K. STRANGE. Effect of cell swelling on membrane and cytoplasmic distribution of pICln. *Am. J. Physiol.* 274 (*Cell Physiol.* 43): C1545—C1551, 1998.
106. EMMA, F., M. McMANUS, AND K. STRANGE. Intracellular electrolytes regulate the volume set point of the organic osmolyte/anion channel VSOAC. *Am. J. Physiol.* 272 (*Cell Physiol.* 41): C1766—C1775, 1997.
107. ESCANDE, D., A. COULOMBE, J. F. FAIVRE, E. DEROUBAIX, AND E. CORABOEUF. Two types of transient outward currents in adult human atrial cells. *Am. J. Physiol.* 252 (*Heart Circ. Physiol.* 21): H142—H148, 1987.
108. FAHLKE, C., T. KNITTLE, C. A. GURNETT, K. P. CAMPBELL, AND A. L. GEORGE, JR. Subunit stoichiometry of human muscle chloride channels. *J. Gen. Physiol.* 109: 93–104, 1997.
109. FAHLKE, C., T. H. RHODES, R. R. DESAI, AND A. L. GEORGE, JR. Pore stoichiometry of a voltage-gated chloride channel. *Nature* 394: 687–690, 1998.
110. FAHLKE, C., H. T. YU, C. L. BECK, T. H. RHODES, AND A. L. GEORGE, JR. Pore-forming segments in voltage-gated chloride channels. *Nature* 390: 529–532, 1997.
111. FAIVRE, J. F., AND A. BRIL. The cardiac chloride channels as molecular targets for antiarrhythmic therapy. *Pharmacol. Res. Commun.* 9: 61–70, 1997.
112. FAIVRE, J. F., S. ROUANET, AND A. BRIL. Comparative effects of glibenclamide, tedisamil, dofetilide, E-4031, and BRL-32872 on protein kinase A-activated chloride current in guinea-pig ventricular mycoytes. *J. Cardiovasc. Pharmacol.* 31: 551–557, 1999.
113. FEDIDA, D., A. P. BRAUN, AND W. R. GILES. Alpha$_1$-adrenoceptors in myocardium: functional aspects and transmembrane signaling mechanisms. *Physiol. Rev.* 73: 469–487, 1993.
114. FISCHER, H., B. ILLEK, AND T. E. MACHEN. Regulation of CFTR by protein phosphatase 2B and protein kinase C. *Pflügers Arch.* 436: 175–181, 1998.
115. FISCHER, H., AND T. E. MACHEN. CFTR displays voltage dependence and two gating modes during stimulation. *J. Gen. Physiol.* 104: 541–566, 1994.
116. FISCHER, H., AND T. E. MACHEN. The tyrosine kinase p60$^{c\text{-}src}$ regulates the fast gate of the cystic fibrosis transmembrane conductance regulator chloride channel. *Biophys. J.* 41: 3073–3082, 1996.
117. FISHER, S. E., G. C. BLACK, S. E. LLOYD, E. HATCHWELL, O. WRONG, R. V. THAKKER, AND I. W. CRAIG. Isolation and partial characterization of a chloride channel gene which is expressed in kidney and is a candidate for Dent's disease (an X-linked hereditary nephrolithiasis). *Hum. Mol. Genet.* 3: 2053–2059, 1994.
118. FLENS, M. J., G. J. ZAMAN, D. V. VAN, M. A. IZQUIERDO, A. B. SCHROEIJERS, G. L. SCHEFFER, D. G. VAN, H. M. DE, C. J. MEIJER, AND R. J. SCHEPER. Tissue distribution of the multidrug resistance protein. *Am. J. Pathol.* 148: 1237–1247, 1996.
119. FORTE, M., E. BLACHLY-DYSON, AND M. COLOMBINI. Structure and function of the yeast outer mitochondrial membrane channel, VDAC. *Soc. Gen. Physiol. Ser.* 51: 145–154, 1996.
120. FOSKETT, J. K. ClC and CFTR chloride channel gating. *Annu. Rev. Physiol.* 60: 689–717, 1998.
121. FRACE, A. M., AND H. C. HARTZELL. Opposite effects of phosphatase inhibitors on L-type calcium and delayed rectifier currents in frog cardiac myocytes. *J. Physiol. (Lond.)* 472: 305–326, 1993.
122. FRACE, A. M., F. MARUOKA, AND A. NOMA. Control of the hyperpolarization-activated cation current by external anions in rabbit sino-atrial node cells. *J. Physiol. (Lond.)* 453: 307–318, 1992.
123. FRACE, A. M., P. F. MERY, R. FISCHMEISTER, AND H. C. HARTZELL. Rate-limiting steps in the beta-adrenergic stimulation of cardiac calcium current. *J. Gen. Physiol.* 101: 337–353, 1993.
124. FRANZ, M. R. Mechano-electrical feedback in ventricular myocardium. *Cardiovasc. Res.* 32: 15–24, 1996.
125. FRANZ, M. R., R. CIMA, D. WANG, D. PROFITT, AND R. KURZ. Electrophysiological effects of myocardial stretch and mechanical determinants of stretch-activated arrhythmias. *Circulation* 86: 968–978, 1992.
126. FRENCH, P. J., J. BIJMAN, A. G. BOT, W. E. BOOMAARS, B. J. SCHOLTE, AND J. H. DE. Genistein activates CFTR Cl$^-$ channels via a tyrosine kinase- and protein phosphatase-independent mechanism. *Am. J. Physiol.* 273 (*Cell Physiol.* 42): C747—C753, 1997.
127. FRIZZELL, R. A. The molecular physiology of cyctic fibrosis. *News Physiol. Sci.* 8: 117–120, 1993.
128. FUJINAGA, J., X.-B. TANG, AND J. R. CASEY. Topology of the membrane domain of human erythrocyte anion exchange protein, AE1. *J. Biol. Chem.* 274: 6626–6633, 1999.
129. FULLER, C. M., AND D. J. BENOS. CFTR! *Am. J. Physiol.* 263 (*Cell Physiol.* 32): C267—C286, 1992.
130. FURUKAWA, T., S. HORIKAWA, T. TERAI, T. OGURA, Y. KATAYAMA, AND M. HIRAOKA. Molecular cloning and characterization of a novel truncated from (ClC-2 beta) of ClC-2 alpha (ClC-2G) in rabbit heart. *FEBS Lett.* 375: 56–62, 1995.
131. FURUKAWA, T., R. J. MYERBURG, N. FURUKAWA, A. L. BASSETT, AND S. KIMURA. Differences in transient outward currents of feline endocardial and epicardial myocytes. *Circ. Res.* 67: 1287–1291, 1990.
132. FURUKAWA, T., T. OGURA, Y. KATAYAMA, AND M. HIRAOKA. Characteristics of rabbit ClC-2 current expressed in *Xenopus* oocytes and its contribution to volume regulation. *Am. J. Physiol.* 274 (*Cell Physiol.* 43): C500—C512, 1998.
133. GADSBY, D. C. Two-bit anion channel really shapes up. *Nature* 383: 295–296, 1996.
134. GADSBY, D. C., T. C. HWANG, T. BAUKROWITZ, G. NAGEL, M. HORIE, AND A. C. NAIRN. Regulation of CFTR channel gating. *Jpn. J. Physiol.* 44, *Suppl.* 2: S183—S192, 1994.
135. GADSBY, D. C., T. C. HWANG, M. HORIE, G. NAGEL, AND A. C. NAIRN. Cardiac chloride channels: incremental regulation by phosphorylation/dephosphorylation. *Ann. NY Acad. Sci.* 707: 259–274, 1993.
136. GADSBY, D. C., G. NAGEL, AND T. C. HWANG. The CFTR chloride channel of mammalian heart. *Annu. Rev. Physiol.* 57: 387–416, 1995.
137. GADSBY, D. C., AND A. C. NAIRN. Regulation of CFTR channel gating. *Trends Biochem. Sci.* 19: 513–518, 1994.
138. GADSBY, D. C., AND A. C. NAIRN. Control of cystic fibrosis transmembrane conductance regulator channel gating by phosphorylation and nucleotide hydrolysis. *Physiol. Rev.* 79: 1–31, 1999.
139. GANDHI, R., R. C. ELBLE, A. D. GRUBER, K. D. SCHREUR, H. L. JI, C. M. FULLER, AND B. U. PAULI. Molecular and functional characterization of a calcium-sensitive chloride channel from mouse lung. *J. Biol. Chem.* 273: 32096–32101, 1998.
140. GAO, T., A. YATANI, M. L. DELL'ACQUA, H. SAKO, S. A. GREEN, N. DASCAL, J. D. SCOTT, AND M. M. HOSEY. cAMP-dependent regulation of cardiac L-type Ca^{2+} channels requires membrane targeting of PKA and phosphorylation of channel subunits. *Neuron* 19: 185–196, 1997.
141. GARBER, S., AND M. D. CAHALAN. Volume-regulated anion channels and the control of a simple cell behavior. *Cell. Physiol. Biochem.* 7: 229–241, 1997.
142. GOPAL, R., AND E. R. TEN EICK. Cytochalasin D alters relationship between selling-activated chloride current and cardiac myocyte size (Abstract). *Biophys. J.* 72: A226, 1997.
143. GRAY, P. C., B. D. JOHNSON, R. E. WESTENBROEK, L. G. HAYS, J. R. YATES, T. SCHEUER, W. A. CATTERALL, AND B. J. MURPHY. Primary structure and function of an A kinase anchoring protein associated with calcium channels. *Neuron* 20: 1017–1026, 1998.
144. GRINSTEIN, S., W. FURUYA, AND L. BIANCHINI. Protein kinases, phosphatases, and the control of cell volume. *News Physiol. Sci.* 7: 232–237, 1992.
145. GRONEMEIER, M., A. CONDIE, J. PROSSER, K. STEINMEYER, T. J. JENTSCH, AND H. JOCKUSCH. Nonsense and missense mutations in the muscular chloride channel gene Clc-1 of myotonic mice. *J. Biol. Chem.* 269: 5963–5967, 1994.
146. GRUBER, A. D., R. C. ELBLE, H. L. JI, K. D. SCHREUR, C. M. FULLER, AND B. U. PAULI. Genomic cloning, molecular characterization, and functional analysis of human CLCA1, the first human member of the family of Ca^{2+}-activated Cl$^-$ channel proteins. *Genomics* 54: 200–214, 1998.

147. GRUNDER, S., A. THIEMANN, M. PUSCH, AND T. J. JENTSCH. Regions involved in the opening of CIC-2 chloride channel by voltage and cell volume. *Nature* 360: 759–762, 1992.
148. GSCHWENTNER, M., U. O. NAGL, E. WOLL, A. SCHMARDA, M. RITTER, AND M. PAULMICHL. Antisense oligonucleotides suppress cell-volume-induced activation of chloride channels. *Pflügers Arch.* 430: 464–470, 1995.
149. GUARINO, R. D., D. M. PEREZ, AND M. T. PIASCIK. Recent advances in the molecular pharmacology of the alpha$_1$-adrenergic receptors. *Cell Signal.* 8: 323–333, 1996.
150. GUNDERSON, K. L., AND R. R. KOPITO. Effects of pyrophosphate and nucleotide analogs suggest a role for ATP hydrolysis in cystic fibrosis transmembrane regulator channel gating. *J. Biol. Chem.* 269: 19349–19353, 1994.
151. GUNDERSON, K. L., AND R. R. KOPITO. Conformational states of CFTR associated with channel gating: the role ATP binding and hydrolysis. *Cell* 82: 231–239, 1995.
152. HAAS, M. The Na-K-Cl cotransporters. *Am. J. Physiol.* 267 (*Cell Physiol.* 36): C869—C885, 1994.
153. HAGIWARA, N., H. MASUDA, M. SHODA, AND H. IRISAWA. Stretch-activated anion currents of rabbit cardiac myocytes. *J. Physiol. (Lond.)* 456: 285–302, 1992.
154. HALL, S. K., J. ZHANG, AND M. LIEBERMAN. Cyclic AMP prevents activation of a swelling-induced chloride-sensitive conductance in chick heart cells. *J. Physiol. (Lond.)* 488: 359–369, 1995.
155. HALM, D. R. Identifying swelling-activated channels from ion selectivity patterns. *J. Gen. Physiol.* 112: 369–371, 1998.
156. HAN, J., C. LEEM, I. SO, E. KIM, S. HONG, W. HO, H. SUNG, AND Y. E. EARM. Effects of thyroid hormone on the calcium current and isoprenaline-induced background current in rabbit ventricular myocytes. *J. Mol. Cell. Cardiol.* 26: 925–935, 1994.
157. HAN, X., AND G. R. FERRIER. Ionic mechanisms of transient inward current in the absence of Na^+- Ca^{2+} exchange in rabbit cardiac Purkinje fibres. *J. Physiol. (Lond.)* 456: 19–38, 1992.
158. HART, P., J. D. WARTH, P. C. LEVESQUE, M. L. COLLIER, Y. GEARY, B. HOROWITZ, AND J. R. HUME. Cystic fibrosis gene encodes a cAMP-dependent chloride channel in heart. *Proc. Natl. Acad. Sci. USA* 93: 6343–6348, 1996.
159. HARTZELL, H. C. Regulation of cardiac ion channels by catecholamines, acetylcholine and second messenger systems. *Prog. Biophys. Mol. Biol.* 52: 165–247, 1988.
160. HARTZELL, H. C., Y. HIRAYAMA, AND J. PETIT-JACQUES. Effects of protein phosphatase and kinase inhibitors on the cardiac L-type Ca current suggest two sites are phosphorylated by protein kinase A and another protein kinase. *J. Gen. Physiol.* 106: 393–414, 1995.
161. HARVEY, R. D. Effects of stilbenedisulfonic acid derivatives on the cAMP-regulated chloride current in cardiac myocytes. *Pflügers Arch.* 422: 436–442, 1993.
162. HARVEY, R. D. Cardiac chloride currents. *News Physiol. Sci.* 11: 175–181, 1996.
163. HARVEY, R. D., C. D. CLARK, AND J. R. HUME. Chloride current in mammalian cardiac myocytes. Novel mechanism for autonomic regulation of action potential duration and resting membrane potential. *J. Gen. Physiol.* 95: 1077–1102, 1990.
164. HARVEY, R. D., AND J. R. HUME. Autonomic regulation of a chloride current in heart. *Science* 244: 983–985, 1989.
165. HARVEY, R. D., AND J. R. HUME. Isoproterenol activates a chloride current, not the transient outward current, in rabbit ventricular myocytes. *Am. J. Physiol.* 257 (*Cell Physiol.* 26): C1177—C1181, 1989.
166. HARVEY, R. D., AND J. R. HUME. Histamine activates the chloride current in cardiac ventricular myocytes. *J. Cardiovasc. Electrophysiol.* 1: 309–317, 1990.
167. HARVEY, R. D., J. A. JUREVICIUS, AND J. R. HUME. Intracellular Na^+ modulates the cAMP-dependent regulation of ion channels in the heart. *Proc. Natl. Acad. Sci. USA* 88: 6946–6950, 1991.
168. HERZIG, S., P. PATIL, J. NEUMANN, C. M. STASCHEN, AND D. T. YUE. Mechanisms of beta-adrenergic stimulation of cardiac Ca^{2+} channels revealed by discrete-time Markov analysis of slow gating. *Biophys. J.* 65: 1599–1612, 1993.
169. HESCHELER, J., M. KAMEYAMA, W. TRAUTWEIN, G. MIESKES, AND H. D. SOLING. Regulation of the cardiac calcium channel by protein phosphatases. *Eur. J. Biochem.* 165: 261–266, 1987.
170. HIGGINS, C. F. The ABC of channel regulation. *Cell* 82: 693–696, 1995.
171. HIGGINS, C. F., K. H. WEYLANDT, C. NASTRUCCI, A. SARDINI, K. LINTON, M. DIAZ, AND M. A. VALVERDE. Cell swelling-activated chloride channels and their regulation by P-glycoprotein. In: *Chloride Channels*, edited by R. Kozlowski. Oxford, UK: Isis Medical Media. In press.
172. HILL, J. A. J., R. CORONADO, AND H. C. STRAUSS. Reconstitution of ionic channels from human heart. *J. Mol. Cell. Cardiol.* 21: 315–322, 1989.
173. HIRAOKA, M., AND S. KAWANO. Calcium-sensitive and -insensitive transient outward current in rabbit ventricular myocytes. *J. Physiol. (Lond.)* 410: 187–212, 1989.
174. HIRAOKA, M., S. KAWANO, Y. HIRANO, AND T. FURUKAWA. Role of cardiac chloride currents in changes in action potential characteristics and arrhythmias. *Cardiovasc. Res.* 40: 23–33, 1998.
175. HIRAYAMA, Y., AND H. C. HARTZELL. Effects of protein phosphatase and kinase inhibitors on Ca^{2+} and Cl^- currents in guinea pig ventricular myocytes. *Mol. Pharmacol.* 52: 725–734, 1997.
176. HONG-LONG, J., M. D. DUVALL, H. K. PATTON, C. L. SATTERFIELD, C. M. FULLER, AND D. J. BENOS. Functional expression of a truncated Ca^{2+}-activated Cl^- channel and activation by phorbol ester. *Am. J. Physiol.* 274 (*Cell Physiol.* 43): C455—C464, 1998.
177. HOOL, L. C., AND R. D. HARVEY. Role of beta$_1$- and beta$_2$-adrenergic receptors in regulation of Cl^- and Ca^{2+} channels in guinea pig ventricular myocytes. *Am. J. Physiol.* 273 (*Heart Circ. Physiol.* 42): H1669—H1676, 1997.
178. HOOL, L. C., L. M. MIDDLETON, AND R. D. HARVEY. Genistein increases the sensitivity of cardiac ion channels to beta- adrenergic receptor stimulation. *Circ. Res.* 83: 33–42, 1998.
179. HOOL, L. C., L. M. OLEKSA, AND R. D. HARVEY. Role of G proteins in alpha$_1$-adrenergic inhibition of the beta- adrenergically activated chloride current in cardiac myocytes. *Mol. Pharmacol.* 51: 853–860, 1997.
180. HORIE, M., T. C. HWANG, AND D. C. GADSBY. Pipette GTP is essential for receptor-mediated regulation of Cl^- current in dialysed myocytes from guinea-pig ventricle. *J. Physiol. (Lond.)* 455: 235–246, 1992.
181. HOROWITZ, B., D. DUAN, G. M. DICK, J. YAMAZAKI, AND J. R. HUME. Volume-regulated chloride channels in heart and smooth muscle. In: *Chloride Channels*, edited by R. Kozlowski. Oxford, UK: Isis Medical Media. In press.
182. HOROWITZ, B., S. S. TSUNG, P. HART, P. C. LEVESQUE, AND J. R. HUME. Alternative splicing of CFTR Cl^- channels in heart. *Am. J. Physiol.* 264 (*Heart Circ. Physiol.* 33): H2214—H2220, 1993.
183. HOSHI, T., W. N. ZAGOTTA, AND R. W. ALDRICH. Biophysical and molecular mechanisms of *Shaker* potassium channel inactivation. *Science* 250: 533–538, 1990.
184. HU, H., AND F. SACHS. Stretch-activated ion channels in the heart. *J. Mol. Cell. Cardiol.* 29: 1511–1523, 1997.
185. HUME, J. R., P. HART, P. C. LEVESQUE, M. L. COLLIER, Y. GEARY, J. WARTH, T. CHAPMAN, AND B. HOROWITZ. Molecular physiology of CFTR Cl^- channels in heart. *Jpn. J. Physiol.* 44, *Suppl.* 2: S177—S182, 1994.
186. HUME, J. R., AND R. D. HARVEY. Chloride conductance pathways in heart. *Am. J. Physiol.* 261 (*Cell Physiol.* 30): C399—C412, 1991.
187. HUME, J. R., AND B. HOROWITZ. A plethora of cardiac chloride conductances: molecular diversity or a related gene family. *J. Cardiovasc. Electrophysiol.* 6: 325–331, 1995.
188. HUTTER, O. F., AND D. NOBLE. Anion conductance of cardiac muscle. *J. Physiol. (Lond.)* 157: 335–350, 1961.
189. HWANG, T. C., AND D. C. GADSBY. Chloride ion channels in mammalian heart cells. In: *Current Topics in Membranes.* Orlando, FL: Academic, 1994, p. 317–346.
190. HWANG, T. C., M. HORIE, AND D. C. GADSBY. Functionally distinct phospho-forms underlie incremental activation of protein kinase-regulated Cl^- conductance in mammalian heart. *J. Gen. Physiol.* 101: 629–650, 1993.
191. HWANG, T. C., M. HORIE, A. C. NAIRN, AND D. C. GADSBY. Role of GTP-binding proteins in the regulation of mammalian cardiac chloride conductance. *J. Gen. Physiol.* 99: 465–489, 1992.
192. HWANG, T. C., G. NAGEL, A. C. NAIRN, AND D. C. GADSBY. Regulation of the gating of cystic fibrosis transmembrane conduc-

tance regulator C1 channels by phosphorylation and ATP hydrolysis. *Proc. Natl. Acad. Sci. USA* 91: 4698–4702, 1994.
193. ILLEK, B., H. FISCHER, AND T. E. MACHEN. Alternate stimulation of apical CFTR by genistein in epithelia. *Am. J. Physiol.* 270 (*Cell Physiol.* 39): C265—C275, 1996.
194. ILLEK, B., H. FISCHER, G. F. SANTOS, J. H. WIDDICOMBE, T. E. MACHEN, AND W. W. REENSTRA. cAMP-independent activation of CFTR Cl channels by the tyrosine kinase inhibitor genistein. *Am. J. Physiol.* 268 (*Cell Physiol.* 37): C886—C893, 1995.
195. ISHIHARA, H., AND M. J. WELSH. Block by MOPS reveals a conformation change in the CFTR pore produced by ATP hydrolysis. *Am. J. Physiol.* 273 (*Cell Physiol.* 42): C1278—C1289, 1997.
196. IYADOMI, I., K. HIRAHARA, AND T. EHARA. α-Adrenergic inhibition of the beta-adrenoceptor-dependent chloride current in guinea-pig ventricular myocytes. *J. Physiol. (Lond.)* 489: 95–104, 1995.
197. JACKSON, P. S., AND K. STRANGE. Single-channel properties of a volume-sensitive anion conductance. Current activation occurs by abrupt switching of closed channels to an open state. *J. Gen. Physiol.* 105: 643–660, 1995.
198. JAMES, A. F., T. TOMINAGA, Y. OKADA, AND M. TOMINAGA. Distribution of cAMP-activated chloride current and CFTR mRNA in the guinea pig heart. *Circ. Res.* 79: 201–207, 1996.
199. JAMES, A. F., L. H. XIE, Y. FUJITANI, S. HAYASHI, AND M. HORIE. Inhibition of the cardiac protein kinase A-dependent chloride conductance by endothelin-1. *Nature* 370: 297–300, 1994.
200. JANUARY, C. T., AND H. A. FOZZARD. Delayed afterdepolarizations in heart muscle: mechanisms and relevance. *Pharmacol. Rev.* 40: 219–227, 1988.
201. JENNINGS, M. L. Structure and function of the red blood cell anion transport protein. *Annu. Rev. Biophys. Biophys. Chem.* 18: 397–430, 1989.
202. JENNINGS, M. L., AND N. AL-ROHIL. Kinetics of activation and inactivation of swelling-stimulated K^+/Cl^- transport. The volume-sensitive parameter is the rate constant for inactivation. *J. Gen. Physiol.* 95: 1021–1040, 1990.
203. JENNINGS, M. L., AND R. K. SCHULZ. Okadaic acid inhibition of KCl cotransport. Evidence that protein dephosphorylation is necessary for activation of transport by either cell swelling or *N*-ethylmaleimide. *J. Gen. Physiol.* 97: 799–817, 1991.
204. JENNINGS, R. B., K. A. REIMER, AND C. STEENBERGEN. Myocardial ischemia revisited. The osmolar load, membrane damage, and reperfusion. *J. Mol. Cell. Cardiol.* 18: 769–780, 1986.
205. JENTSCH, T. J. Chloride channels: a molecular perspective. *Curr. Opin. Neurobiol.* 6: 303–310, 1996.
206. JENTSCH, T. J., AND W. GÜNTHER. Chloride channels: an emerging molecular picture. *Bioessays* 19: 117–126, 1997.
207. JENTSCH, T. J., W. GUNTHER, M. PUSCH, AND B. SCHWAPPACH. Properties of voltage-gated chloride channels of the ClC gene family. *J. Physiol. (Lond.)* 482: 19S—25S, 1995.
208. JENTSCH, T. J., K. STEINMEYER, AND G. SCHWARZ. Primary structure of *Torpedo marmorata* chloride channel isolated by expression cloning in *Xenopus* oocytes. *Nature* 348: 510–514, 1990.
209. JIA, Y., C. J. MATHEWS, AND J. W. HANRAHAN. Phosphorylation by protein kinase C is required for acute activation of cystic fibrosis transmembrane conductance regulator by protein kinase A. *J. Biol. Chem.* 272: 4978–4984, 1997.
210. JORDT, S. E., AND T. J. JENTSCH. Molecular dissection of gating in the ClC-2 chloride channel. *EMBO J.* 16: 1582–1592, 1997.
211. KAMEYAMA, M., J. HESCHELER, G. MIESKES, AND W. TRAUTWEIN. The protein-specific phosphatase 1 antagonizes the beta-adrenergic increase of the cardiac Ca current. *Pflügers Arch.* 407: 461–463, 1986.
212. KANEDA, M., K. FUKUI, AND K. DOI. Activation of chloride current by P_2-purinoceptors in rat ventricular myocytes. *Br. J. Pharmacol.* 111: 1355–1360, 1994.
213. KAPLAN, M. R., D. B. MOUNT, E. DELPIRE, G. GAMBA, AND S. C. HEBERT. Molecular mechanisms of NaCl cotransport. *Annu. Rev. Physiol.* 58: 649–668, 1996.
214. KASS, R. S., W. J. LEDERER, R. W. TSIEN, AND R. WEINGART. Role of calcium ions in transient inward currents and aftercontractions induced by strophanthidin in cardiac Purkinje fibres. *J. Physiol. (Lond.)* 281: 187–208, 1978.
215. KAWANO, S., AND M. HIRAOKA. Transient outward currents and action potential alterations in rabbit ventricular myocytes. *J. Mol. Cell. Cardiol.* 23: 681–693, 1991.
216. KAWANO, S., AND M. HIRAOKA. Protein kinase A-activated chloride channel is inhibited by the Ca^{2+}-calmodulin complex in cardiac sarcoplasmic reticulum. *Circ. Res.* 73: 751–757, 1993.
217. KAWANO, S., Y. HIRAYAMA, AND M. HIRAOKA. Activation mechanism of Ca^{2+}-sensitive transient outward current in rabbit ventricular myocytes. *J. Physiol. (Lond.)* 486: 593–604, 1995.
218. KAWANO, S., F. NAKAMURA, T. TANAKA, AND M. HIRAOKA. Cardiac sarcoplasmic reticulum chloride channels regulated by protein kinase A. *Circ. Res.* 71: 585–589, 1992.
219. KAWASAKI, M., M. SUZUKI, S. UCHIDA, S. SASAKI, AND F. MARUMO. Stable and functional expression of the ClC-3 chloride channel in somatic cell lines. *Neuron* 14: 1285–1291, 1995.
220. KAWASAKI, M., S. UCHIDA, T. MONKAWA, A. MIYAWAKI, K. MIKOSHIBA, F. MARUMO, AND S. SASAKI. Cloning and expression of a protein kinase C-regulated chloride channel abundantly expressed in rat brain neuronal cells. *Neuron* 12: 597–604, 1994.
221. KEATING, M. T., AND M. C. SANGUINETTI. Molecular genetic insights into cardiovascular disease. *Science* 272: 681–685, 1996.
222. KEENAN, C., AND D. KELLEHER. Protein kinase C and the cytoskeleton. *Cell. Signal.* 10: 225–232, 1998.
223. KENYON, J. L., AND W. R. GIBBONS. 4-Aminopyridine and the early outward current of sheep cardiac Purkinje fibers. *J. Gen. Physiol.* 73: 139–157, 1979.
224. KENYON, J. L., AND W. R. GIBBONS. Influence of chloride, potassium, and tetraethylammonium on the early outward current of sheep cardiac Purkinje fibers. *J. Gen. Physiol.* 73: 117–138, 1979.
225. KIMURA, J., S. MIYAMAE, AND A. NOMA. Identification of sodium-calcium exchange current in single ventricular cells of guinea-pig. *J. Physiol. (Lond.)* 384: 199–222, 1987.
226. KINNALLY, K. W., D. B. ZOROV, Y. N. ANTONENKO, S. H. SNYDER, M. W. MCENERY, AND H. TEDESCHI. Mitochondrial benzodiazepine receptor linked to inner membrane ion channels by nanomolar actions of ligands. *Proc. Natl. Acad. Sci. USA* 90: 1374–1378, 1993.
227. KIRK, J., AND K. KIRK. Inhibition of volume-activated I^- and taurine efflux from HeLa cells by P-glycoprotein blockers correlates with calmodulin inhibition. *J. Biol. Chem.* 269: 29389–29394, 1994.
228. KLOCKNER, U. Intracellular calcium ions activate a low-conductance chloride channel in smooth-muscle cells isolated from human mesenteric artery. *Pflügers Arch.* 424: 231–237, 1993.
229. KOCH, M. C., K. STEINMEYER, C. LORENZ, K. RICKER, F. WOLF, M. OTTO, B. ZOLL, F. LEHMANN-HORN, K. H. GRZESCHIK, AND T. J. JENTSCH. The skeletal muscle chloride channel in dominant and recessive human myotonia. *Science* 257: 797–800, 1992.
230. KONARZEWSKA, H., G. A. PEETERS, AND M. C. SANGUINETTI. Repolarizing K^+ currents in nonfailing human hearts. Similarities between right septal subendocardial and left subepicardial ventricular myocytes. *Circulation* 92: 1179–1187, 1995.
231. KOWDLEY, G. C., S. J. ACKERMAN, Z. CHEN, G. SZABO, L. R. JONES, AND J. R. MOORMAN. Anion, cation, and zwitterion selectivity of phospholemman channel molecules. *Biophys. J.* 72: 141–145, 1997.
232. KOWDLEY, G. C., S. J. ACKERMAN, J. E. JOHN, L. R. JONES, AND J. R. MOORMAN. Hyperpolarization-activated chloride currents in *Xenopus* oocytes. *J. Gen. Physiol.* 103: 217–230, 1994.
233. KRAPIVINSKY, G. B., M. J. ACKERMAN, E. A. GORDON, L. D. KRAPIVINSKY, AND D. E. CLAPHAM. Molecular characterization of a swelling-induced chloride conductance regulatory protein, pICln. *Cell* 76: 439–448, 1994.
234. KUBO, M., AND Y. OKADA. Volume-regulatory Cl^- channel currents in cultured human epithelial cells. *J. Physiol. (Lond.)* 456: 351–371, 1992.
235. KUDRYCKI, K. E., P. R. NEWMAN, AND G. E. SHULL. cDNA cloning and tissue distribution of mRNAs for two proteins that are related to the band 3 Cl^-/HCO_3^- exchanger. *J. Biol. Chem.* 265: 462–471, 1990.
236. KURUMA, A., M. HIRAOKA, AND S. KAWANO. Activation of Ca^{2+}-sensitive Cl^- current by reverse mode Na^+/Ca^{2+} exchange in rabbit ventricular myocytes. *Pflügers Arch.* 436: 976–983, 1998.
237. LAB, M. J. Mechanoelectric feedback (transduction) in heart: concepts and implications. *Cardiovasc. Res.* 32: 3–14, 1996.

238. LAFLAMME, M. A., AND P. L. BECKER. Ca^{2+}-induced current oscillations in rabbit ventricular myocytes. *Circ. Res.* 78: 707–716, 1996.
239. LANG, F., G. L. BUSCH, M. RITTER, H. VOLKL, S. WALDEGGER, E. GULBINS, AND D. HAUSSINGER. Functional significance of cell volume regulatory mechanisms. *Physiol. Rev.* 78: 247–306, 1998.
240. LARGE, W. A., AND Q. WANG. Characteristics and physiological role of the Ca^{2+}-activated Cl^- conductance in smooth muscle. *Am. J. Physiol.* 271 (*Cell Physiol.* 40): C435—C454, 1996.
241. LARSEN, A. K., B. S. JENSEN, AND E. K. HOFFMANN. Activation of protein kinase C during cell volume regulation in Ehrlich mouse ascites tumor cells. *Biochim. Biophys. Acta* 1222: 477–482, 1994.
242. LAUF, P. K. KCl cotransport: sulfhydryls, divalent cations and the mechanism of volume activation in a red cell. *J. Membr. Biol.* 88: 1–13, 1985.
243. LEBLAIS, V., S. DEMOLOMBE, G. VALLETTE, D. LANGIN, I. BARO, D. ESCANDE, AND C. GAUTHIER. β_3-Adrenoceptor control the cystic fibrosis transmembrane conductance regulator through a cAMP/protein kinase A-independent pathway. *J. Biol. Chem.* 274: 6107–6113, 1999.
244. LEDERER, W. J., E. NIGGLI, AND R. W. HADLEY. Sodium-calcium exchange in excitable cells: fuzzy space. *Science* 248: 283, 1990.
245. LEE, M. A., R. C. DUNN, D. E. CLAPHAM, AND L. STEHNO-BITTEL. Calcium regulation of nuclear pore permeability. *Cell Calcium* 23: 91–101, 1998.
246. LEEM, C., AND R. D. VAUGHAN-JONES. Chloride-hydroxyl exchange in the guinea-pig ventricular myocyte: no role for bicarbonate. *J. Mol. Cell. Cardiol.* 29: 2483–2489, 1997.
247. LEEM, C. H., W. K. HO, AND Y. E. EARM. The effect of taurine on the activation osmolality of the osmosensitive current in single ventricular myocytes of rabbits. *Exp. Physiol.* 81: 189–202, 1996.
248. LEGSSYER, A., J. POGGIOLI, D. RENARD, AND G. VASSORT. ATP and other adenine compounds increase mechanical activity and inositol trisphosphate production in rat heart. *J. Physiol. (Lond.)* 401: 185–199, 1988.
249. LEHMANN-HORN, F., AND R. RUDEL. Channelopathies: their contribution to our knowledge about voltage-gated ion channels. *News Physiol. Sci.* 12: 105–112, 1997.
250. LEVESQUE, P. C., C. D. CLARK, S. I. ZAKAROV, L. V. ROSENSHTRAUKH, AND J. R. HUME. Anion and cation modulation of the guinea-pig ventricular action potential during beta-adrenoceptor stimulation. *Pflügers Arch.* 424: 54–62, 1993.
251. LEVESQUE, P. C., P. J. HART, J. R. HUME, J. L. KENYON, AND B. HOROWITZ. Expression of cystic fibrosis transmembrane regulator Cl^- channels in heart. *Circ. Res.* 71: 1002–1007, 1992.
252. LEVESQUE, P. C., AND J. R. HUME. ATP_o but not $cAMP_i$ activates a chloride conductance in mouse ventricular myocytes. *Cardiovasc. Res.* 29: 336–343, 1995.
253. LEVITAN, I., AND S. S. GARBER. Anion competition for a volume-regulated current. *Biophys. J.* 75: 226–235, 1998.
254. LI, G. R., J. FENG, Z. WANG, B. FERMINI, AND S. NATTEL. Comparative mechanisms of 4-aminopyridine-resistant I_{to} in human and rabbit atrial myocytes. *Am. J. Physiol.* 269 (*Heart Circ. Physiol.* 38): H463—H472, 1995.
255. LI, G. R., J. FENG, Z. WANG, AND S. NATTEL. Transmembrane chloride currents in human atrial myocytes. *Am. J. Physiol.* 270 (*Cell Physiol.* 39): C500—C507, 1996.
256. LINN, S. C., G. R. ASKEW, A. G. MENON, AND G. E. SHULL. Conservation of an AE3 Cl^-/HCO_3^- exchanger cardiac-specific exon and promoter region and AE3 mRNA expression patterns in murine and human hearts. *Circ. Res.* 76: 584–591, 1995.
257. LINN, S. C., K. E. KUDRYCKI, AND G. E. SHULL. The predicted translation product of a cardiac AE3 mRNA contains an N terminus distinct from that of the brain AE3 Cl^-/HCO_3^- exchanger. Cloning of a cardiac AE3 cDNA, organization of the AE3 gene, and identification of an alternative transcription initiation site. *J. Biol. Chem.* 267: 7927–7935, 1992.
258. LINSDELL, P., AND J. W. HANRAHAN. Flickery block of single CFTR chloride channels by intracellular anions and osmolytes. *Am. J. Physiol.* 271 (*Cell Physiol.* 40): C628—C634, 1996.
259. LINSDELL, P., AND J. W. HANRAHAN. Adenosine triphosphate-dependent asymmetry of anion permeation in the cystic fibrosis transmembrane conductance regulator chloride channel. *J. Gen. Physiol.* 111: 601–614, 1998.
260. LIPP, P., AND E. NIGGLI. Submicroscopic calcium signals as fundamental events of excitation-contraction coupling in guinea-pig cardiac myocytes. *J. Physiol. (Lond.)* 492: 31–38, 1996.
261. LIU, S., R. JACOB, D. PIWNICA-WORMS, AND M. LIEBERMAN. (Na-K-2Cl) cotransport in cultured embryonic chick heart cells. *Am. J. Physiol.* 253 (*Cell Physiol.* 22): C721—C730, 1987.
262. LIU, S., AND M. LIEBERMAN. Electrodiffusive movements of chloride ion in sodium-free solution: a possible calcium activated chloride conductance in cultured heart cells. *Cardiovasc. Res.* 28: 1629–1634, 1994.
263. LIU, S., D. PIWNICA-WORMS, AND M. LIEBERMAN. Intracellular pH regulation in cultured embryonic chick heart cells. Na^+-dependent Cl^-/HCO_3^- exchange. *J. Gen. Physiol.* 96: 1247–1269, 1990.
264. LIU, S., J. R. STIMERS, AND M. LIEBERMAN. A novel Cl^- conductance in cultured chick cardiac myocytes: role of intracellular Ca^{2+} and cAMP. *J. Membr. Biol.* 141: 59–68, 1994.
265. LIU, Y., S. OIKI, T. TSUMURA, T. SHIMIZU, AND Y. OKADA. Glibenclamide blocks volume-sensitive Cl^- channels by dual mechanisms. *Am. J. Physiol.* 275 (*Cell Physiol.* 44): C343—C351, 1998.
266. LLOYD, S. E., S. H. PEARCE, S. E. FISHER, K. STEINMEYER, B. SCHWAPPACH, S. J. SCHEINMAN, B. HARDING, A. BOLINO, M. DEVOTO, P. GOODYER, S. P. RIGDEN, O. WRONG, T. J. JENTSCH, I. W. CRAIG, AND R. V. THAKKER. A common molecular basis for three inherited kidney stone diseases. *Nature* 379: 445–449, 1996.
267. LORENZ, C., M. PUSCH, AND T. J. JENTSCH. Heteromultimeric CLC chloride channels with novel properties. *Proc. Natl. Acad. Sci. USA* 93: 13362–13366, 1996.
268. LUCKIE, D. B., M. E. KROUSE, K. L. HARPER, T. C. LAW, AND J. J. WINE. Selection for MDR1/P-glycoprotein enhances swelling-activated K^+ and Cl^- currents in NIH/3T3 cells. *Am. J. Physiol.* 267 (*Cell Physiol.* 36): C650—C658, 1994.
269. LUDEWIG, U., T. J. JENTSCH, AND M. PUSCH. Inward rectification in ClC-0 chloride channels caused by mutations in several protein regions. *J. Gen. Physiol.* 110: 165–171, 1997.
270. LUDEWIG, U., M. PUSCH, AND T. J. JENTSCH. Two physically distinct pores in the dimeric ClC-0 chloride channel. *Nature* 383: 340–343, 1996.
271. LUO, J., M. D. PATO, J. R. RIORDAN, AND J. W. HANRAHAN. Differential regulation of single CFTR channels by PP2C, PP2A, and other phosphatases. *Am. J. Physiol.* 274 (*Cell Physiol.* 43): C1397—C1410, 1998.
272. LYTLE, C. A volume-sensitive protein kinase regulates the Na-K-2Cl cotransporter in duck red blood cells. *Am. J. Physiol.* 274 (*Cell Physiol.* 43): C1002—C1010, 1998.
273. LYTLE, C., AND B. FORBUSH III. The Na-K-Cl cotransport protein of shark rectal gland. II. Regulation by direct phosphorylation. *J. Biol. Chem.* 267: 25438–25443, 1992.
274. MAK, D. O., AND J. K. FOSKETT. Single-channel kinetics, inactivation, and spatial distribution of inositol trisphosphate (IP_3) receptors in *Xenopus* oocyte nucleus. *J. Gen. Physiol.* 109: 571–587, 1997.
275. MALINOWSKA, D. H., E. Y. KUPERT, A. BAHINSKI, A. M. SHERRY, AND J. CUPPOLETTI. Cloning, functional expression, and characterization of a PKA-activated gastric Cl^- channel. *Am. J. Physiol.* 268 (*Cell Physiol.* 37): C191—C200, 1995.
276. MANSOURA, M. K., S. S. SMITH, A. D. CHOI, N. W. RICHARDS, T. V. STRONG, M. L. DRUMM, F. S. COLLINS, AND D. C. DAWSON. Cystic fibrosis transmembrane conductance regulator (CFTR) anion binding as a probe of the pore. *Biophys. J.* 74: 1320–1332, 1998.
277. MARCELL, C. G., M. C. G. DANIELS, D. FEDIDA, C. LAMONT, AND H. E. TER KEURS. Role of sarcolemma in triggered propagated contractions in rat cardiac muscle. *Circ. Res.* 68: 1408–1421, 1998.
278. MARTY, A., Y. P. TAN, AND A. TRAUTMANN. Three types of calcium-dependent channel in rat lacrimal glands. *J. Physiol. (Lond.)* 357: 293–325, 1984.
279. MARUNAKA, Y., AND D. C. EATON. Effects of insulin and phosphatase on a Ca^{2+}-dependent Cl^- channel in a distal nephron cell line (A6). *J. Gen. Physiol.* 95: 773–789, 1990.
280. MATSUOKA, S., T. EHARA, AND A. NOMA. Chloride-sensitive na-

ture of the adrenaline-induced current in guinea-pig cardiac myocytes. *J. Physiol. (Lond.)* 425: 579–598, 1990.
281. MATSUOKA, S., A. NOMA, AND T. POWELL. Li^+ inhibition of membrane current responses to epinephrine in guinea-pig ventricular cells. *Pflügers Arch.* 415: 384–386, 1989.
282. MATSUURA, H., AND T. EHARA. Activation of chloride current by purinergic stimulation in guinea pig heart cells. *Circ. Res.* 70: 851–855, 1992.
283. MATTHEWS, G., E. NEHER, AND R. PENNER. Chloride conductance activated by external agonists and internal messengers in rat peritoneal mast cells. *J. Physiol. (Lond.)* 418: 131–144, 1989.
284. MAYLIE, J., AND M. MORAD. A transient outward current related to calcium release and development of tension in elephant seal atrial fibres. *J. Physiol. (Lond.)* 357: 267–292, 1984.
285. MAZZANTI, M., L. J. DEFELICE, J. COHN, AND H. MALTER. Ion channels in the nuclear envelope. *Nature* 343: 764–767, 1990.
286. MCCANN, J. D., M. LI, AND M. J. WELSH. Identification and regulation of whole-cell chloride currents in airway epithelium. *J. Gen. Physiol.* 94: 1015–1036, 1989.
287. MCDONALD, T. F., S. PELZER, W. TRAUTWEIN, AND D. J. PELZER. Regulation and modulation of calcium channels in cardiac, skeletal, and smooth muscle cells. *Physiol. Rev.* 74: 365–507, 1994.
288. MCDONOUGH, S., N. DAVIDSON, H. A. LESTER, AND N. A. MCCARTY. Novel pore-lining residues in CFTR that govern permeation and open-channel block. *Neuron* 13: 623–634, 1994.
289. MENG, X. J., AND S. A. WEINMAN. cAMP- and swelling-activated chloride conductance in rat hepatocytes. *Am. J. Physiol.* 271 (*Cell Physiol.* 40): C112—C120, 1996.
290. MIDDLETON, L. M., AND R. D. HARVEY. PKC regulation of cardiac CFTR Cl^- channel function in guinea pig ventricular myocytes. *Am. J. Physiol.* 275 (*Cell Physiol.* 44): C293—C302, 1998.
291. MIDDLETON, R. E., D. J. PHEASANT, AND C. MILLER. Homodimeric architecture of a ClC-type chloride ion channel. *Nature* 383: 337–340, 1996.
292. MILLER, C., AND M. M. WHITE. Dimeric structure of single chloride channels from *Torpedo electroplax*. *Proc. Natl. Acad. Sci. USA* 81: 2772–2775, 1984.
293. MINTON, A. P., G. C. COLCLASURE, AND J. C. PARKER. Model for the role of macromolecular crowding in regulation of cellular volume. *Proc. Natl. Acad. Sci. USA* 89: 10504–10506, 1992.
294. MOHAMMADI, K., P. ROUET-BENZINEB, M. LAPLACE, AND B. CROZATIER. Protein kinase C activity and expression in rabbit left ventricular hypertrophy. *J. Mol. Cell. Cardiol.* 29: 1687–1694, 1997.
295. MONROE, R. G., F. C. LA, W. J. GAMBLE, R. P. HAMMOND, AND C. L. MORGAN. Norepinephrine release and left ventricular pressure in the isolated heart. *Circ. Res.* 19: 774–790, 1966.
296. MOORMAN, J. R., S. J. ACKERMAN, G. C. KOWDLEY, M. P. GRIFFIN, J. P. MOUNSEY, Z. CHEN, S. E. CALA, J. J. O'BRIAN, G. SZABO, AND L. R. JONES. Unitary anion currents through phospholemman channel molecules. *Nature* 377: 737–740, 1995.
297. MOORMAN, J. R., AND L. R. JONES. Phospholemman: a cardiac taurine channel involved in regulation of cell volume. *Adv. Exp. Med. Biol.* 442: 219–228, 1998.
298. MOORMAN, J. R., C. J. PALMER, J. E. JOHN, M. E. DURIEUX, AND L. R. JONES. Phospholemman expression induces a hyperpolarization-activated chloride current in *Xenopus* oocytes. *J. Biol. Chem.* 267: 14551–14554, 1992.
299. MORIN, X. K., T. D. BOND, T. W. LOO, D. M. CLARKE, AND C. E. BEAR. Failure of P-glycoprotein (*MDR1*) expressed in *Xenopus* oocytes to produce swelling-activated chloride channel activity. *J. Physiol. (Lond.)* 486: 707–714, 1995.
300. MORITA, H., J. KIMURA, AND M. ENDOH. Angiotensin II activation of a chloride current in rabbit cardiac myocytes. *J. Physiol. (Lond.)* 483: 119–130, 1995.
301. MUSCH, M. W., E. M. DAVIS-AMARAL, H. H. VANDENBURGH, AND L. GOLDSTEIN. Hypotonicity stimulates translocation of ICln in neonatal rat cardiac myocytes. *Pflügers Arch.* 436: 415–422, 1998.
302. NABAUER, M., D. J. BEUCKELMANN, AND E. ERDMANN. Characteristics of transient outward current in human ventricular myocytes from patients with terminal heart failure. *Circ. Res.* 73: 386–394, 1993.
303. NABAUER, M., D. J. BEUCKELMANN, P. UBERFUHR, AND G. STEINBECK. Regional differences in current density and rate-dependent properties of the transient outward current in subepicardial and subendocardial myocytes of human left ventricle. *Circulation* 93: 168–177, 1996.
304. NAGEL, G., T. C. HWANG, K. L. NASTIUK, A. C. NAIRN, AND D. C. GADSBY. The protein kinase A-regulated cardiac Cl^- channel resembles the cystic fibrosis transmembrane conductance regulator. *Nature* 360: 81–84, 1992.
305. NAKAJIMA, T., T. SUGIMOTO, AND Y. KURACHI. Effects of anions on the G protein-mediated activation of the muscarinic K^+ channel in the cardiac atrial cell membrane. *J. Gen. Physiol.* 99: 665–682, 1999.
306. NAKASHIMA, Y., AND K. ONO. Rate-limiting steps in activation of cardiac Cl^- current revealed by photolytic application of cAMP. *Am. J. Physiol.* 267 (*Heart Circ. Physiol.* 36): H1514—H1522, 1994.
307. NAKAYAMA, T., AND H. A. FOZZARD. Adrenergic modulation of the transient outward current in isolated canine Purkinje cells. *Circ. Res.* 62: 162–172, 1988.
308. NELSON, M. T. Bayliss, myogenic tone and volume-regulated chloride channels in arterial smooth muscle. *J. Physiol. (Lond.)* 507: 629–629, 1998.
309. NELSON, M. T., M. A. CONWAY, H. J. KNOT, AND J. E. BRAYDEN. Chloride channel blockers inhibit myogenic tone in rat cerebral arteries. *J. Physiol. (Lond.)* 502: 259–264, 1997.
310. NGUYEN, T., W.-C. CHIN, AND P. VERDUGO. Role of Ca^{2+}/K^+ ion exchange in intracellular storage and release of Ca^{2+}. *Nature* 395: 908–912, 1998.
311. NICHOLS, C. G., AND W. J. LEDERER. Adenosine triphosphate-sensitive potassium channels in the cardiovascular system. *Am. J. Physiol.* 261 (*Heart Circ. Physiol.* 30): H1675—H1686, 1991.
312. NICHOLS, C. G., AND A. N. LOPATIN. Inward rectifier potassium channels. *Annu. Rev. Physiol.* 59: 171–191, 1997.
313. NILIUS, B., J. EGGERMONT, T. VOETS, AND G. DROOGMANS. Volume-activated Cl^- channels. *Gen. Pharmacol.* 27: 1131–1140, 1996.
314. NILIUS, B., J. PRENEN, T. VOETS, J. EGGERMONT, AND G. DROOGMANS. Activation of volume-regulated chloride currents by reduction of intracellular ionic strength in bovine endothelial cells. *J. Physiol. (Lond.)* 506: 353–361, 1998.
315. NILIUS, B., T. VOETS, J. PRENEN, H. BARTH, K. AKTORIES, K. KAIBUCHI, G. DROOGMANS, AND J. EGGERMONT. Role of rho and rho-kinase in the activation of volume-regulated anion channels in bovine endothelial cells. *J. Physiol. (Lond.)* 516: 67–74, 1999.
316. NOMA, A., AND H. IRISAWA. Membrane currents in the rabbit sinoatrial node cell as studied by the double microelectrode method. *Pflügers Arch.* 364: 45–52, 1976.
317. OKADA, Y. Volume expansion-sensing outward-rectifier Cl^- channel: fresh start to the molecular identity and volume sensor. *Am. J. Physiol.* 273 (*Cell Physiol.* 42): C755—C789, 1997.
318. OKADA, Y., M. KUBO, S. OIKI, C. C. PETERSEN, M. TOMINAGA, A. HAZAMA, AND S. MORISHIMA. Properties of volume-sensitive Cl^- channels in a human epithelial cell line. *Jpn. J. Physiol.* 44, *Suppl.* 2: S31—S35, 1994.
319. OKADA, Y., S. OIKI, A. HAZAMA, AND S. MORISHIMA. Criteria for the molecular identification of the volume-sensitive outwardly rectifying Cl^- channel. *J. Gen. Physiol.* 112: 365–367, 1998.
320. OKADA, Y., S. OIKI, M. TOMINAGA, M. KUBO, A. MIWA, T. TOMINAGA, T. TSUMURA, AND K. UEDA. Volume-sensitive Cl^- channel in human epithelial cells: regulation by ATP and relation to P-glycoprotein. *Jpn. J. Physiol.* 47, *Suppl.* 1: S19—S20, 1997.
321. OLEKSA, L. M., L. C. HOOL, AND R. D. HARVEY. $Alpha_1$-adrenergic inhibition of the beta-adrenergically activated Cl^- current in guinea pig ventricular myocytes. *Circ. Res.* 78: 1090–1099, 1996.
322. ONO, K., AND H. A. FOZZARD. Phosphorylation restores activity of L-type calcium channels after rundown in inside-out patches from rabbit cardiac cells. *J. Physiol. (Lond.)* 454: 673–688, 1992.
323. ONO, K., AND A. NOMA. Autonomic regulation of cardiac chloride current. *Jpn. J. Physiol.* 44, *Suppl.* 2: S193—S198, 1994.
324. ONO, K., F. M. TAREEN, A. YOSHIDA, AND A. NOMA. Synergistic action of cyclic GMP on catecholamine-induced chloride current in guinea-pig ventricular cells. *J. Physiol. (Lond.)* 453: 647–661, 1992.
325. OVERHOLT, J. L., M. E. HOBERT, AND R. D. HARVEY. On the mechanism of rectification of the isoproterenol-activated chloride

current in guinea-pig ventricular myocytes. *J. Gen. Physiol.* 102: 871–895, 1993.
326. OVERHOLT, J. L., A. SAULINO, M. L. DRUMM, AND R. D. HARVEY. Rectification of whole cell cystic fibrosis transmembrane conductance regulator chloride current. *Am. J. Physiol.* 268 (*Cell Physiol.* 37): C636—C646, 1995.
327. OZ, M. C., AND S. SOROTA. Forskolin stimulates swelling-induced chloride current, not cardiac cystic fibrosis transmembrane-conductance regulator current, in human cardiac myocytes. *Circ. Res.* 76: 1063–1070, 1995.
328. PALFREY, H. C. Protein phosphorylation control in the activity of volume-sensitive transport systems. In: *Cellular and Molecular Physiology of Cell Volume Regulation*, edited by K. Strange. Boca Raton, FL: CRC, 1994, p. 201–214.
329. PALMER, C. J., B. T. SCOTT, AND L. R. JONES. Purification and complete sequence determination of the major plasma membrane substrate for cAMP-dependent protein kinase and protein kinase C in myocardium. *J. Biol. Chem.* 266: 11126–11130, 1991.
330. PAPP, Z., K. R. SIPIDO, G. CALLEWAERT, AND E. CARMELIET. Two components of $[Ca^{2+}]_i$-activated Cl^- current during large $[Ca^{2+}]_i$ transients in single rabbit heart Purkinje cells. *J. Physiol. (Lond.)* 483: 319–330, 1995.
331. PARKER, J. C., G. C. COLCLASURE, AND T. J. MCMANUS. Coordinated regulation of shrinkage-induced Na/H exchange and swelling-induced KCl cotransport in dog red cells. *J. Gen. Physiol.* 98: 869–880, 1991.
332. PATTERSON, E., J. J. LYNCH, AND B. R. LUCCHESI. Antiarrhythmic and antifibrillatory actions of the beta adrenergic receptor antagonist, DL-sotalol. *J. Pharmacol. Exp. Ther.* 230: 519–526, 1984.
333. PAULMICHL, M., Y. LI, K. WICKMAN, M. ACKERMAN, E. PERALTA, AND D. CLAPHAM. New mammalian chloride channel identified by expression cloning. *Nature* 356: 238–241, 1992.
334. PELZER, S., Y. YOU, Y. M. SHUBA, AND D. J. PELZER. Beta-adrenoceptor-coupled G_s protein facilitates the activation of cAMP-dependent cardiac Cl^- current. *Am. J. Physiol.* 273 (*Heart Circ. Physiol.* 42): H2539—H2548, 1997.
335. PICCIOTTO, M. R., J. A. COHN, G. BERTUZZI, P. GREENGARD, AND A. C. NAIRN. Phosphorylation of the cystic fibrosis transmembrane conductance regulator. *J. Biol. Chem.* 267: 12742–12752, 1992.
336. PIWNICA-WORMS, D., R. JACOB, C. R. HORRES, AND M. LIEBERMAN. Potassium-chloride cotransport in cultured chick heart cells. *Am. J. Physiol.* 249 (*Cell Physiol.* 18): C337—C344, 1985.
337. PRAT, A. G., Y. F. XIAO, D. A. AUSIELLO, AND H. F. CANTIELLO. cAMP-independent regulation of CFTR by the actin cytoskeleton. *Am. J. Physiol.* 268 (*Cell Physiol.* 37): C1552—C1561, 1995.
338. PRICE, M. P., H. ISHIHARA, D. N. SHEPPARD, AND M. J. WELSH. Function of *Xenopus* cystic fibrosis transmembrane conductance regulator (CFTR) Cl channels and use of human-*Xenopus* chimeras to investigate the pore properties of CFTR. *J. Biol. Chem.* 271: 25184–25191, 1996.
339. PUCEAT, M., C. BONY, M. JACONI, AND G. VASSORT. Specific activation of adenylyl cyclase V by a purinergic agonist. *FEBS Lett.* 431: 189–194, 1998.
340. PUCEAT, M., O. CLEMENT, AND G. VASSORT. Extracellular MgATP activates the Cl^-/HCO_3^- exchanger in single rat cardiac cells. *J. Physiol. (Lond.)* 444: 241–256, 1991.
341. PUCEAT, M., I. KORICHNEVA, R. CASSOLY, AND G. VASSORT. Identification of band 3-like proteins and Cl^-/HCO_3^- exchange in isolated cardiomyocytes. *J. Biol. Chem.* 270: 1315–1322, 1995.
342. PUSCH, M., AND T. J. JENTSCH. Molecular physiology of voltage-gated chloride channels. *Physiol. Rev.* 74: 813–827, 1994.
343. PUSCH, M., U. LUDEWIG, A. REHFELDT, AND T. J. JENTSCH. Gating of the voltage-dependent chloride channel CIC-0 by the permeant anion. *Nature* 373: 527–531, 1995.
344. RANKIN, A. C., R. SITSAPESAN, AND K. A. KANE. Antagonism by adenosine and ATP of an isoprenaline-induced background current in guinea-pig ventricular myocytes. *J. Mol. Cell. Cardiol.* 22: 1371–1378, 1990.
345. RASMUSSON, R. L., D. G. DAVIS, AND M. LIEBERMAN. Amino acid loss during volume regulatory decrease in cultured chick heart cells. *Am. J. Physiol.* 264 (*Cell Physiol.* 33): C136—C145, 1993.
346. REDDY, M. M., P. M. QUINTON, C. HAWS, J. J. WINE, R. GRYGORCZYK, J. A. TABCHARANI, J. W. HANRAHAN, K. L. GUNDERSON, AND R. R. KOPITO. Failure of the cystic fibrosis transmembrane conductance regulator to conduct ATP. *Science* 271: 1876–1879, 1996.
347. REENSTRA, W. W., K. YURKO-MAURO, A. DAM, S. RAMAN, AND S. SHORTEN. CFTR chloride channel activation by genistein: the role of serine/threonine protein phosphatases. *Am. J. Physiol.* 271 (*Cell Physiol.* 40): C650—C657, 1996.
348. REES, S. A., J. I. VANDENBERG, A. R. WRIGHT, A. YOSHIDA, AND T. POWELL. Cell swelling has differential effects on the rapid and slow components of delayed rectifier potassium current in guinea pig cardiac myocytes. *J. Gen. Physiol.* 106: 1151–1170, 1995.
349. REISIN, I. L., A. G. PRAT, E. H. ABRAHAM, J. F. AMARA, R. J. GREGORY, D. A. AUSIELLO, AND H. F. CANTIELLO. The cystic fibrosis transmembrane conductance regulator is a dual ATP and chloride channel. *J. Biol. Chem.* 269: 20584–20591, 1994.
350. RICHARDS, S. M., M. E. JACONI, G. VASSORT, AND M. PUCEAT. A spliced variant of AE1 gene encodes a truncated form of band 3 in heart: the predominant anion exchanger in ventricular myocytes. *J. Cell Sci. Suppl.* 112: 1519–1528, 1999.
351. RIDLEY, P. D., AND M. J. CURTIS. Anion manipulation: a new antiarrhythmic approach. Action of substitution of chloride with nitrate on ischemia- and reperfusion-induced ventricular fibrillation and contractile function. *Circ. Res.* 70: 617–632, 1992.
352. RIEMER, T. L., E. A. SOBIE, AND L. TUNG. Stretch-induced changes in arrhythmogenesis and excitability in experimentally based heart cell models. *Am. J. Physiol.* 275 (*Heart Circ. Physiol.* 44): H431—H442, 1998.
353. RIORDAN, J. R. The cystic fibrosis transmembrane conductance regulator. *Annu. Rev. Physiol.* 55: 609–630, 1993.
354. RIORDAN, J. R., J. M. ROMMENS, B. KEREM, N. ALON, R. ROZMAHEL, Z. GRZELCZAK, J. ZIELENSKI, S. LOK, N. PLAVSIC, AND J. L. CHOU. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 245: 1066–1073, 1989.
355. ROBERTSON, M. A., AND J. K. FOSKETT. Na^+ transport pathways in secretory acinar cells: membrane cross-talk mediated by $[Cl^-]_i$. *Am. J. Physiol.* 267 (*Cell Physiol.* 36): C146—C156, 1994.
356. ROBSON, L., AND M. HUNTER. Role of cell volume and protein kinase C in regulation of a Cl^- conductance in single proximal tubule cells of *Rana temporaria*. *J. Physiol. (Lond.)* 480: 1–7, 1994.
357. ROMMENS, J. M., M. C. IANNUZZI, B. KEREM, M. L. DRUMM, G. MELMER, M. DEAN, R. ROZMAHEL, J. L. COLE, D. KENNEDY, AND N. HIDAKA. Identification of the cystic fibrosis gene: chromosome walking and jumping. *Science* 245: 1059–1065, 1989.
358. ROSTOVTSEVA, T., AND M. COLOMBINI. VDAC channels mediate and gate the flow of ATP: implications for the regulation of mitochondrial function. *Biophys. J.* 72: 1954–1962, 1997.
359. ROUET-BENZINEB, P., K. MOHAMMADI, J. PERENNEC, M. POYARD, N. BOUANANI, AND B. CROZATIER. Protein kinase C isoform expression in normal and failing rabbit hearts. *Circ. Res.* 79: 153–161, 1996.
360. ROUSSEAU, E. Single chloride-selective channel from cardiac sarcoplasmic reticulum studied in planar lipid bilayers. *J. Membr. Biol.* 110: 39–47, 1989.
361. ROUSSEAU, E., C. MICHAUD, D. LEFEBVRE, S. PROTEAU, AND A. DECROUY. Reconstitution of ionic channels from inner and outer membranes of mammalian cardiac nuclei. *Biophys. J.* 70: 703–714, 1996.
362. RUIZ, P. E., Z. A. PONCE, AND O. F. SCHANNE. Early action potential shortening in hypoxic hearts: role of chloride current(s) mediated by catecholamine release. *J. Mol. Cell Cardiol.* 28: 279–290, 1996.
363. SACHS, F. Modeling mechanical-electrical transduction in the heart. In: *Cell Mechanics and Cellular Engineering*, edited by V. C. Mow, F. Guliak, R. Tran-Son-Tray, and R. Hochmuth. New York: Springer, 1994, p. 308–328.
364. SADOSHIMA, J., Z. QIU, J. P. MORGAN, AND S. IZUMO. Tyrosine kinase activation is an immediate and essential step in hypotonic cell swelling-induced ERK activation and c-*fos* gene expression in cardiac myocytes. *EMBO J.* 15: 5535–5546, 1996.
365. SAKAGUCHI, M., H. MATSUURA, AND T. EHARA. Swelling-induced Cl^- current in guinea-pig atrial myocytes: inhibition by glibenclamide. *J. Physiol. (Lond.)* 505: 41–52, 1997.

366. SAKAI, R., N. HAGIWARA, H. KASANUKI, AND S. HOSODA. Chloride conductance in human atrial cells. *J. Mol. Cell. Cardiol.* 27: 2403–2408, 1995.
367. SAKAMOTO, H., M. KAWASAKI, S. UCHIDA, S. SASAKI, AND F. MARUMO. Identification of a new outwardly rectifying Cl^- channel that belongs to a subfamily of the ClC Cl^- channels. *J. Biol. Chem.* 271: 10210–10216, 1996.
368. SANGUINETTI, M. C., N. K. JURKIEWICZ, A. SCOTT, AND P. K. SIEGL. Isoproterenol antagonizes prolongation of refractory period by the class III antiarrhythmic agent E-4031 in guinea pig myocytes. *Circ. Res.* 68: 77–84, 1991.
369. SASAKI, N., T. MITSUIYE, AND A. NOMA. Effects of mechanical stretch on membrane currents of single ventricular myocytes of guinea-pig heart. *Jpn. J. Physiol.* 42: 957–970, 1992.
370. SASAKI, N., T. MITSUIYE, Z. WANG, AND A. NOMA. Increase of the delayed rectifier K^+ and Na^+-K^+ pump currents by hypotonic solutions in guinea pig cardiac myocytes. *Circ. Res.* 75: 887–895, 1994.
371. SATO, R., AND S. KOUMI. Characterization of the stretch-activated chloride channel in isolated human atrial myocytes. *J. Membr. Biol.* 163: 67–76, 1998.
372. SCHMIDT-ROSE, T., AND T. J. JENTSCH. Transmembrane topology of a CLC chloride channel. *Proc. Natl. Acad. Sci. USA* 94: 7633–7638, 1997.
373. SCHULTZ, B. D., A. K. SINGH, D. C. DEVOR, AND R. J. BRIDGES. Pharmacology of CFTR chloride channel activity. *Physiol. Rev.* 79, *Suppl.*: S109—S144, 1999.
374. SCHWIEBERT, E. M., L. P. CID-SOTO, D. STAFFORD, M. CARTER, C. J. BLAISDELL, P. L. ZEITLIN, W. B. GUGGINO, AND G. R. CUTTING. Analysis of ClC-2 channels as an alternative pathway for chloride conduction in cystic fibrosis airway cells. *Proc. Natl. Acad. Sci. USA* 95: 3879–3884, 1998.
375. SCHWIEBERT, E. M., M. E. EGAN, T. H. HWANG, S. B. FULMER, S. S. ALLEN, G. R. CUTTING, AND W. B. GUGGINO. CFTR regulates outwardly rectifying chloride channels through an autocrine mechanism involving ATP. *Cell* 81: 1063–1073, 1995.
376. SEARS, C. L., F. FIROOZMAND, A. MELLANDER, F. G. CHAMBERS, I. G. EROMAR, A. G. BOT, B. SCHOLTE, H. R. DEJONGE, AND M. DONOWITZ. Genistein and tyrophostin 47 stimulate CFTR-mediated Cl^- secretion in T84 cell monolayers. *Am. J. Physiol.* 269 (*Gastrointest. Liver Physiol.* 32): G874—G882, 1995.
377. SEIBERT, F. S., P. LINSDELL, T. W. LOO, J. W. HANRAHAN, D. M. CLARKE, AND J. R. RIORDAN. Disease-associated mutations in the fourth cytoplasmic loop of cystic fibrosis transmembrane conductance regulator compromise biosynthetic processing and chloride channel activity. *J. Biol. Chem.* 271: 15139–15145, 1996.
378. SEIBERT, F. S., P. LINSDELL, T. W. LOO, J. W. HANRAHAN, J. R. RIORDAN, AND D. M. CLARKE. Cytoplasmic loop three of cystic fibrosis transmembrane conductance regulator contributes to regulation of chloride channel activity. *J. Biol. Chem.* 271: 27493–27499, 1996.
379. SEIBERT, F. S., T. W. LOO, D. M. CLARKE, AND J. R. RIORDAN. Cystic fibrosis: channel, catalytic, and folding properties of the CFTR protein. *J. Bioenerg. Biomembr.* 29: 429–442, 1997.
380. SENIOR, A. E., AND D. C. GADSBY. ATP hydrolysis cycles and mechanism in P-glycoprotein and CFTR. *Semin. Cancer Biol.* 8: 143–150, 1997.
381. SEYAMA, I. Characteristics of the anion channel in the sino-atrial node cell of the rabbit. *J. Physiol. (Lond.)* 294: 447–460, 1979.
382. SHEPPARD, D. N., AND K. A. ROBINSON. Mechanism of glibenclamide inhibition of cystic fibrosis transmembrane conductance regulator Cl^- channels expressed in a murine cell line. *J. Physiol. (Lond.)* 503: 333–346, 1997.
383. SHEPPARD, D. N., AND M. J. WELSH. Effect of ATP-sensitive K^+ channel regulators on cystic fibrosis transmembrane conductance regulator chloride currents. *J. Gen. Physiol.* 100: 573–591, 1992.
384. SHEPPARD, D. N., AND M. J. WELSH. Structure and function of the CFTR chloride channel. *Physiol. Rev.* 79, *Suppl.*: S23—S45, 1999.
385. SHERRY, A. M., K. STROFFEKOVA, L. M. KNAPP, E. Y. KUPERT, J. CUPPOLETTI, AND D. H. MALINOWSKA. Characterization of the human pH- and PKA-activated ClC-2G(2 alpha) Cl^- channel. *Am. J Physiol* 273 (*Cell Physiol.* 42): C384—C393, 1997.
386. SHIDA, S., H. NAKAYA, AND M. KANNO. Effects of Cl^- channel blockers on beta-adrenoceptor-mediated decreases in resting potential and intracellular Cl^- activity in guinea-pig heart. *Eur. J. Pharmacol.* 212: 267–270, 1992.
387. SHUBA, L. M., T. ASAI, AND T. F. MCDONALD. Phorbol ester activation of chloride current in guinea-pig ventricular myocytes. *Br. J. Pharmacol.* 117: 1395–1404, 1996.
388. SHUBA, L. M., T. ASAI, S. PELZER, AND T. F. MCDONALD. Activation of cardiac chloride conductance by the tyrosine kinase inhibitor, genistein. *Br. J. Pharmacol.* 119: 335–345, 1996.
389. SHUBA, L. M., AND T. F. MCDONALD. Synergistic activation of guinea-pig cardiac cystic fibrosis transmembrane conductance regulator by the tyrosine kinase inhibitor genistein and cAMP. *J. Physiol. (Lond.)* 505: 23–40, 1997.
390. SHUBA, L. M., T. OGURA, AND T. F. MCDONALD. Kinetic evidence distinguishing volume-sensitive chloride current from other types in guinea-pig ventricular myocytes. *J. Physiol. (Lond.)* 491: 69–80, 1996.
391. SIEGELBAUM, S. A., AND R. W. TSIEN. Calcium-activated transient outward current in calf cardiac Purkinje fibres. *J. Physiol. (Lond.)* 299: 485–506, 1980.
392. SIMKHOVICH, B. Z., K. PRZYKLENK, AND R. A. KLONER. Role of protein kinase C as a cellular mediator of ischemic preconditioning: a critical review. *Cardiovasc. Res.* 40: 9–22, 1998.
393. SIPIDO, K. R., G. CALLEWAERT, AND E. CARMELIET. $[Ca^{2+}]_i$ transients and $[Ca^{2+}]_i$-dependent chloride current in single Purkinje cells from rabbit heart. *J. Physiol. (Lond.)* 468: 641–667, 1993.
394. SIPIDO, K. R., G. CALLEWAERT, F. PORCIATTI, J. VEREECKE, AND E. CARMELIET. $[Ca^{2+}]_i$-dependent membrane currents in guinea-pig ventricular cells in the absence of Na/Ca exchange. *Pflügers Arch.* 430: 871–878, 1995.
395. SMIT, L. S., D. J. WILKINSON, M. K. MANSOURA, F. S. COLLINS, AND D. C. DAWSON. Functional roles of the nucleotide-binding folds in the activation of the cystic fibrosis transmembrane conductance regulator. *Proc. Natl. Acad. Sci. USA* 90: 9963–9967, 1993.
396. SNUTCH, T. P., AND P. B. REINER. Ca^{2+} channels: diversity of form and function. *Curr. Opin. Neurobiol.* 2: 247–253, 1992.
397. SOLC, C. K., AND J. J. WINE. Swelling-induced and depolarization-induced Cl^- channels in normal and cystic fibrosis epithelial cells. *Am. J. Physiol.* 261 (*Cell Physiol.* 30): C658—C674, 1991.
398. SORGATO, M. C., AND O. MORAN. Channels in mitochondrial membranes: knowns, unknowns, and prospects for the future. *Crit. Rev. Biochem. Mol. Biol.* 28: 127–171, 1993.
399. SORGATO, M. C., O. MORAN, P. V. DE, B. U. KELLER, AND W. STUEHMER. Further investigation on the high-conductance ion channel of the inner membrane of mitochondria. *J. Bioenerg. Biomembr.* 21: 485–496, 1989.
400. SOROTA, S. Swelling-induced chloride-sensitive current in canine atrial cells revealed by whole-cell patch-clamp method. *Circ. Res.* 70: 679–687, 1992.
401. SOROTA, S. Pharmacologic properties of the swelling-induced chloride current of dog atrial myocytes. *J. Cardiovasc. Electrophysiol.* 5: 1006–1016, 1994.
402. SOROTA, S. Tyrosine protein kinase inhibitors prevent activation of cardiac swelling-induced chloride current. *Pflügers Arch.* 431: 178–185, 1995.
403. SOROTA, S., AND X. Y. DU. Delayed activation of cardiac swelling-induced chloride current after step changes in cell size. *J. Cardiovasc. Electrophysiol.* 9: 825–831, 1998.
404. SOROTA, S., M. S. SIEGAL, AND B. F. HOFFMAN. The isoproterenol-induced chloride current and cardiac resting potential. *J. Mol. Cell. Cardiol.* 23: 1191–1198, 1991.
405. SPITZER, K. W., AND J. L. WALKER. Intracellular chloride activity in quiescent cat papillary muscle. *Am. J Physiol* 238 (*Heart Circ. Physiol.* 7): H487—H493, 1980.
406. STACY, G. P. J., R. L. JOBE, L. K. TAYLOR, AND D. E. HANSEN. Stretch-induced depolarizations as a trigger of arrhythmias in isolated canine left ventricles. *Am. J. Physiol.* 263 (*Heart Circ. Physiol.* 32): H613—H621, 1992.
407. STALEY, K., R. SMITH, J. SCHAACK, C. WILCOX, AND T. J. JENTSCH. Alteration of $GABA_A$ receptor function following gene transfer of the CLC-2 chloride channel. *Neuron* 17: 543–551, 1996.
408. STEHNO-BITTEL, L., A. LUCKHOFF, AND D. E. CLAPHAM. Cal-

cium release from the nucleus by $InsP_3$ receptor channels. *Neuron* 14: 163–167, 1995.
409. STEHNO-BITTEL, L., C. PEREZ-TERZIC, A. LUCKHOFF, AND D. E. CLAPHAM. Nuclear ion channels and regulation of the nuclear pore. *Soc. Gen. Physiol. Ser.* 51: 195–207, 1996.
410. STERN, M. D. Theory of excitation-contraction coupling in cardiac muscle. *Biophys. J.* 63: 497–517, 1992.
411. STRANGE, K. Molecular identity of the outwardly rectifying, swelling-activated anion channel: time to reevaluate pICln. *J. Gen. Physiol.* 111: 617–622, 1998.
412. STRANGE, K., F. EMMA, AND P. S. JACKSON. Cellular and molecular physiology of volume-sensitive anion channels. *Am. J. Physiol.* 270 (*Cell Physiol.* 39): C711—C730, 1996.
413. STROFFEKOVA, K., E. Y. KUPERT, D. H. MALINOWSKA, AND J. CUPPOLETTI. Identification of the pH sensor and activation by chemical modification of the ClC-2G Cl^- channel. *Am. J Physiol* 275 (*Cell Physiol.* 44): C1113—C1123, 1998.
414. STUTTS, M. J., T. C. CHINET, S. J. MASON, J. M. FULLTON, L. L. CLARKE, AND R. C. BOUCHER. Regulation of Cl^- channels in normal and cystic fibrosis airway epithelial cells by extracellular ATP. *Proc. Natl. Acad. Sci. USA* 89: 1621–1625, 1992.
415. STUTTS, M. J., E. R. LAZAROWSKI, A. M. PARADISO, AND R. C. BOUCHER. Activation of CFTR Cl^- conductance in polarized T84 cells by luminal extracellular ATP. *Am. J. Physiol.* 268 (*Cell Physiol.* 37): C425—C433, 1995.
416. SUGDEN, P. H., AND M. A. BOGOYEVITCH. Intracellular signalling through protein kinases in the heart. *Cardiovasc. Res.* 30: 478–492, 1995.
417. SULEYMANIAN, M. A., AND C. M. BAUMGARTEN. Osmotic gradient-induced water permeation across the sarcolemma of rabbit ventricular myocytes. *J. Gen. Physiol.* 107: 503–514, 1996.
418. SULEYMANIAN, M. A., H. F. CLEMO, N. M. COHEN, AND C. M. BAUMGARTEN. Stretch-activated channel blockers modulate cell volume in cardiac ventricular myocytes. *J. Mol. Cell. Cardiol.* 27: 721–728, 1995.
419. SULLIVAN, S. K., K. SWAMY, AND M. FIELD. cAMP-activated Cl conductance is expressed in *Xenopus* oocytes by injection of shark rectal gland mRNA. *Am. J. Physiol.* 260 (*Cell Physiol.* 29): C664—C669, 1991.
420. SUN, B., C. H. LEEM, AND R. D. VAUGHAN-JONES. Novel chloride-dependent acid loader in the guinea-pig ventricular myocyte: part of a dual acid-loading mechanism. *J. Physiol. (Lond.)* 495: 65–82, 1996.
421. SZEWCZYK, A. The intracellular potassium and chloride channels: properties, pharmacology and function. *Mol. Membr. Biol.* 15: 49–58, 1998.
422. TABARES, L., M. MAZZANTI, AND D. E. CLAPHAM. Chloride channels in the nuclear membrane. *J. Membr. Biol.* 123: 49–54, 1991.
423. TABCHARANI, J. A., X. B. CHANG, J. R. RIORDAN, AND J. W. HANRAHAN. Phosphorylation-regulated Cl^- channel in CHO cells stably expressing the cystic fibrosis gene. *Nature* 352: 628–631, 1991.
424. TABCHARANI, J. A., X. B. CHANG, J. R. RIORDAN, AND J. W. HANRAHAN. The cystic fibrosis transmembrane conductance regulator chloride channel. Iodide block and permeation. *Biophys. J.* 62: 1–4, 1992.
425. TABCHARANI, J. A., J. M. ROMMENS, Y. X. HOU, X. B. CHANG, L. C. TSUI, J. R. RIORDAN, AND J. W. HANRAHAN. Multi-ion pore behaviour in the CFTR chloride channel. *Nature* 366: 79–82, 1993.
426. TAKAHASHI, T., E. NEHER, AND B. SAKMANN. Rat brain serotonin receptors in *Xenopus* oocytes are coupled by intracellular calcium to endogenous channels. *Proc. Natl. Acad. Sci. USA* 84: 5063–5067, 1987.
427. TAKANO, M., AND A. NOMA. Distribution of the isoprenaline-induced chloride current in rabbit heart. *Pflügers Arch.* 420: 223–226, 1992.
428. TALEB, O., P. FELTZ, J. L. BOSSU, AND A. FELTZ. Small-conductance chloride channels activated by calcium on cultured endocrine cells from mammalian pars intermedia. *Pflügers Arch.* 412: 641–646, 1988.
429. TANAKA, H., S. MATSUI, T. KAWANISHI, AND K. SHIGENOBU. Use of chloride blockers: a novel approach for cardioprotection against ischemia-reperfusion damage. *J. Pharmacol. Exp. Ther.* 278: 854–861, 1996.
430. TAO, T., J. XIE, M. L. DRUMM, J. ZHAO, P. B. DAVIS, AND J. MA. Slow conversions among subconductance states of cystic fibrosis transmembrane conductance regulator chloride channel. *Biophys. J.* 70: 743–753, 1996.
431. TAOUIL, K., R. GIANCOLA, J. E. MOREL, AND P. HANNAERT. Hypotonically induced calcium increase and regulatory volume decrease in newborn rat cardiomyocytes. *Pflügers Arch.* 436: 565–574, 1998.
432. TAREEN, F. M., K. ONO, A. NOMA, AND T. EHARA. Beta-adrenergic and muscarinic regulation of the chloride current in guinea-pig ventricular cells. *J. Physiol. (Lond.)* 440: 225–241, 1991.
433. TAREEN, F. M., A. YOSHIDA, AND K. ONO. Modulation of beta-adrenergic responses of chloride and calcium currents by external cations in guinea-pig ventricular cells. *J. Physiol. (Lond.)* 457: 211–228, 1992.
434. TERZIC, A., M. PUCEAT, G. VASSORT, AND S. M. VOGEL. Cardiac $alpha_1$-adrenoceptors: an overview. *Pharmacol. Rev.* 45: 147–175, 1993.
435. THIEMANN, A., S. GRUNDER, M. PUSCH, AND T. J. JENTSCH. A chloride channel widely expressed in epithelial and non-epithelial cells. *Nature* 356: 57–60, 1992.
436. TILLY, B. C., K. BEZSTAROSTI, W. E. BOOMAARS, C. R. MARINO, J. M. LAMERS, AND J. H. DE. Expression and regulation of chloride channels in neonatal rat cardiomyocytes. *Mol. Cell. Biochem.* 157: 129–135, 1996.
437. TILLY, B. C., M. J. EDIXHOVEN, L. G. TERTOOLEN, N. MORII, Y. SAITOH, S. NARUMIYA, AND H. R. DE JONGE. Activation of the osmo-sensitive chloride conductance involves P21rho and is accompanied by a transient reorganization of the F-actin cytoskeleton. *Mol. Biol. Cell* 7: 1419–1427, 1996.
438. TILLY, B. C., D. B. VAN, L. G. TERTOOLEN, M. J. EDIXHOVEN, AND H. R. DE JONGE. Protein tyrosine phosphorylation is involved in osmoregulation of ionic conductances. *J. Biol. Chem.* 268: 19919–19922, 1993.
439. TOMINAGA, M., M. HORIE, S. SASAYAMA, AND Y. OKADA. Glibenclamide, an ATP-sensitive K^+ channel blocker, inhibits cardiac cAMP-activated Cl^- conductance. *Circ. Res.* 77: 417–423, 1995.
440. TOMINAGA, M., T. TOMINAGA, A. MIWA, AND Y. OKADA. Volume-sensitive chloride channel activity does not depend on endogenous P-glycoprotein. *J. Biol. Chem.* 270: 27887–27893, 1995.
441. TRAFFORD, A. W., M. E. DIAZ, AND D. A. EISNER. Ca-activated chloride current and Na-Ca exchange have different time courses during sarcoplasmic reticulum Ca release in ferret ventricular myocytes. *Pflügers Arch.* 435: 743–745, 1998.
442. TRAFFORD, A. W., M. E. DIAZ, S. C. O'NEILL, AND D. A. EISNER. Comparison of subsarcolemmal and bulk calcium concentration during spontaneous calcium release in rat ventricular myocytes. *J. Physiol. (Lond.)* 488: 577–586, 1995.
443. TRAVIS, S. M., H. A. BERGER, AND M. J. WELSH. Protein phosphatase 2C dephosphorylates and inactivates cystic fibrosis transmembrane conductance regulator. *Proc. Natl. Acad. Sci. USA* 94: 11055–11060, 1997.
444. TREZISE, A. E. O., K. R. WONG, AND J. I. VANDENBERG. Distribution of CFTR mRNA in rabbit left ventricular myocardium (Abstract). *J. Physiol. (Lond.)* 504: 74P—75P, 1997.
445. TSENG, G. N. Cell swelling increases membrane conductance of canine cardiac cells: evidence for a volume-sensitive Cl channel. *Am. J. Physiol.* 262 (*Cell Physiol.* 31): C1056—C1068, 1992.
446. TSENG, G. N., AND B. F. HOFFMAN. Two components of transient outward current in canine ventricular myocytes. *Circ. Res.* 64: 633–647, 1989.
447. VALVERDE, M. A., M. DIAZ, F. V. SEPULVEDA, D. R. GILL, S. C. HYDE, AND C. F. HIGGINS. Volume-regulated chloride channels associated with the human multidrug-resistance P-glycoprotein. *Nature* 355: 830–833, 1992.
448. VALVERDE, M. A., G. M. MINTENIG, AND F. V. SEPULVEDA. Differential effects of tamoxifen and I^- on three distinguishable chloride currents activated in T84 intestinal cells. *Pflügers Arch.* 425: 552–554, 1993.
449. VANDENBERG, J. I., G. C. BETT, AND T. POWELL. Contribution of a swelling-activated chloride current to changes in the cardiac

action potential. *Am. J. Physiol.* 273 (*Cell Physiol.* 42): C541—C547, 1997.
450. VANDENBERG, J. I., S. A. REES, A. R. WRIGHT, AND T. POWELL. Cell swelling and ion transport pathways in cardiac myocytes. *Cardiovasc. Res.* 32: 85–97, 1996.
451. VANDENBERG, J. I., A. YOSHIDA, K. KIRK, AND T. POWELL. Swelling-activated and isoprenaline-activated chloride currents in guinea pig cardiac myocytes have distinct electrophysiology and pharmacology. *J. Gen. Physiol.* 104: 997–1017, 1994.
452. VAN RENTERGHEM, C., AND M. LAZDUNSKI. Endothelin and vasopressin activate low conductance chloride channels in aortic smooth muscle cells. *Pflügers Arch.* 425: 156–163, 1993.
453. VAN SLEGHTENHORST, M. A., M. T. BASSI, G. BORSANI, M. WAPENAAR, G. B. FERRERO, L. DE CONCILIIS, E. I. RUGARLI, A. GILLO, B. FRANCO, H. Y. ZOGHBI, AND A. BALLABIO. A gene from the Xp22.3 region shares homology with voltage-gated chloride channels. *Hum. Mol. Genet.* 3: 547–552, 1994.
454. VAN WAGONER, D. Mechanosensitive gating of atrial ATP-sensitive potassium channels. *Circ. Res.* 72: 973–983, 1993.
455. VASSORT, G., M. PUCEAT, AND F. SCAMPS. Modulation of myocardial activity by extracellular ATP. *Trends Cardiovasc. Med.* 4: 236–240, 1994.
456. VASSORT, G., F. SCAMPS, M. PUCEAT, AND O. CLEMENT. Multiple site effects of extracellular ATP in cardiac tissues. *News Physiol. Sci.* 7: 212–215, 1992.
457. VAUGHAN-JONES, R. D. Non-passive chloride distribution in mammalian heart muscle: microelectrode measurement of the intracellular chloride activity. *J. Physiol. (Lond.)* 295: 83–109, 1979.
458. VAUGHAN-JONES, R. D. An investigation of chloride-bicarbonate exchange in the sheep cardiac Purkinje fibre. *J. Physiol. (Lond.)* 379: 377–406, 1986.
459. VOETS, T., G. BUYSE, J. TYTGAT, G. DROOGMANS, J. EGGERMONT, AND B. NILIUS. The chloride current induced by expression of the protein pICln in *Xenopus* oocytes differs from the endogenous volume-sensitive chloride current. *J. Physiol. (Lond.)* 495: 441–447, 1996.
460. VOETS, T., V. MANOLOPOULOS, J. EGGERMONT, C. ELLORY, G. DROOGMANS, AND B. NILIUS. Regulation of a swelling-activated chloride current in bovine endothelium by protein tyrosine phosphorylation and G proteins. *J. Physiol. (Lond.)* 506: 341–352, 1998.
461. VON WEIKERSTHAL, S. F., M. A. BARRAND, AND S. B. HLADKY. Functional and molecular characterization of a volume-sensitive chloride current in rat brain endothelial cells. *J. Physiol. (Lond.)* 516: 75–84, 1999.
462. WALDEGGER, S., P. BARTH, G. RABER, AND F. LANG. Cloning and characterization of a putative human serine/threonine protein kinase transcriptionally modified during anisotonic and isotonic alterations of cell volume. *Proc. Natl. Acad. Sci. USA* 94: 4440–4445, 1997.
463. WALSH, K. B. Activation of a heart chloride current during stimulation of protein kinase C. *Mol. Pharmacol.* 40: 342–346, 1991.
464. WALSH, K. B., AND K. J. LONG. Properties of a protein kinase C-activated chloride current in guinea pig ventricular myocytes. *Circ. Res.* 74: 121–129, 1994.
465. WALSH, K. B., AND C. WANG. Effect of chloride channel blockers on the cardiac CFTR chloride and L- type calcium currents. *Cardiovasc. Res.* 32: 391–399, 1996.
466. WALSH, K. B., AND C. WANG. Arylaminobenzoate block of the cardiac cyclic AMP-dependent chloride current. *Mol. Pharmacol.* 53: 539–546, 1998.
467. WANG, F., S. ZELTWANGER, I. C. YANG, A. C. NAIRN, AND T. C. HWANG. Actions of genistein on cystic fibrosis transmembrane conductance regulator channel gating. Evidence for two binding sites with opposite effects. *J. Gen. Physiol.* 111: 477–490, 1998.
468. WANG, L., L. CHEN, V. WALKER, AND T. J. JACOB. Antisense to MDR1 mRNA reduces P-glycoprotein expression, swelling-activated Cl^- current and volume regulation in bovine ciliary epithelial cells. *J. Physiol. (Lond.)* 511: 33–44, 1998.
469. WANG, Y. X., AND M. I. KOTLIKOFF. Inactivation of calcium-activated chloride channels in smooth muscle by calcium/calmodulin-dependent protein kinase. *Proc. Natl. Acad. Sci. USA* 94: 14918–14923, 1997.
470. WANG, Z., T. MITSUIYE, S. A. REES, AND A. NOMA. Regulatory volume decrease of cardiac myocytes induced by beta-adrenergic activation of the Cl^- channel in guinea pig. *J. Gen. Physiol.* 110: 73–82, 1997.
471. WARTH, J. D., M. L. COLLIER, P. HART, Y. GEARY, C. H. GELBAND, T. CHAPMAN, B. HOROWITZ, AND J. R. HUME. CFTR chloride channels in human and simian heart. *Cardiovasc. Res.* 31: 615–624, 1996.
472. WARTH, J. D., AND J. R. HUME. Re-examination of Na^+-dependent regulation of cAMP-dependent Cl^- currents in the heart. *Pflügers Arch.* 433: 597–607, 1997.
473. WEBB, T. E., M. O. BOLUYT, AND E. A. BARNARD. Molecular biology of P_{2Y} purinoceptors: expression in rat heart. *J. Auton. Pharmacol.* 16: 303–307, 1996.
474. WEINREICH, F., P. G. WOOD, J. R. RIORDAN, AND G. NAGEL. Direct action of genistein on CFTR. *Pflügers Arch.* 434: 484–491, 1997.
475. WELSH, M. J. Abnormal regulation of ion channels in cystic fibrosis epithelia. *FASEB J.* 4: 2718–2725, 1990.
476. WELSH, M. J., AND B. W. RAMSEY. Research on cystic fibrosis. A journey from the heart house. *Am. J. Respir. Crit. Care Med.* 157: S148—S154, 1998.
477. WELSH, M. J., AND A. E. SMITH. Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. *Cell* 73: 1251–1254, 1993.
478. WELSH, M. J., AND A. E. SMITH. Cystic fibrosis. *Sci. Am.* 273: 52–59, 1995.
479. WETTWER, E., G. AMOS, J. GATH, H. R. ZERKOWSKI, J. C. REIDEMEISTER, AND U. RAVENS. Transient outward current in human and rat ventricular myocytes. *Cardiovasc. Res.* 27: 1662–1669, 1993.
480. WETTWER, E., G. J. AMOS, H. POSIVAL, AND U. RAVENS. Transient outward current in human ventricular myocytes of subepicardial and subendocardial origin. *Circ. Res.* 75: 473–482, 1994.
481. WHITE, M. M., AND M. AYLWIN. Niflumic and flufenamic acids are potent reversible blockers of Ca^{2+}-activated Cl^- channels in *Xenopus* oocytes. *Mol. Pharmacol.* 37: 720–724, 1990.
482. WILKINSON, D. J., M. K. MANSOURA, P. Y. WATSON, L. S. SMIT, F. S. COLLINS, AND D. C. DAWSON. CFTR: the nucleotide binding folds regulate the accessibility and stability of the activated state. *J. Gen. Physiol.* 107: 103–119, 1996.
483. WILKINSON, D. J., T. V. STRONG, M. K. MANSOURA, D. L. WOOD, S. S. SMITH, F. S. COLLINS, AND D. C. DAWSON. CFTR activation: additive effects of stimulatory and inhibitory phosphorylation sites in the R domain. *Am. J. Physiol.* 273 (*Lung Cell. Mol. Physiol.* 17): L127—L133, 1997.
484. WINE, J. J., AND D. B. LUCKIE. Cell-volume regulation: P-glycoprotein–a cautionary tale. *Curr. Biol.* 6: 1410–1412, 1996.
485. WINPENNY, J. P., H. L. MCALROY, M. A. GRAY, AND B. E. ARGENT. Protein kinase C regulates the magnitude and stability of CFTR currents in pancreatic duct cells. *Am. J. Physiol.* 268 (*Cell Physiol.* 37): C823—C828, 1995.
486. WOLFF, A. A., AND R. LEVI. Histamine and cardiac arrhythmias. *Circ. Res.* 58: 1–16, 1986.
487. WORRELL, R. T., A. G. BUTT, W. H. CLIFF, AND R. A. FRIZZELL. A volume-sensitive chloride conductance in human colonic cell line T84. *Am. J. Physiol.* 256 (*Cell Physiol.* 25): C1111—C1119, 1989.
488. WRIGHT, A. R., AND S. A. REES. Targeting ischaemia–cell swelling and drug efficacy. *Trends Pharmacol. Sci.* 18: 224–228, 1997.
489. WRIGHT, A. R., S. A. REES, J. I. VANDENBERG, V. W. TWIST, AND T. POWELL. Extracellular osmotic pressure modulates sodium-calcium exchange in isolated guinea-pig ventricular myocytes. *J. Physiol. (Lond.)* 488: 293–301, 1995.
490. WRIGHT, E. M., AND J. M. DIAMOND. Anion selectivity in biological systems. *Physiol. Rev.* 57: 109–156, 1977.
491. WU, G., AND O. P. HAMILL. NPPB block of Ca^{2+}-activated Cl^- currents in *Xenopus* oocytes. *Pflügers Arch.* 420: 227–229, 1992.
492. XIE, J., M. L. DRUMM, J. MA, AND P. B. DAVIS. Intracellular loop between transmembrane segments IV and V of cystic fibrosis transmembrane conductance regulator is involved in regulation of chloride channel conductance state. *J. Biol. Chem.* 270: 28084–28091, 1995.
493. XIE, J., M. L. DRUMM, J. ZHAO, J. MA, AND P. B. DAVIS. Human epithelial cystic fibrosis transmembrane conductance regulator

without exon 5 maintains partial chloride channel function in intracellular membranes. *Biophys. J.* 71: 3148–3156, 1996.
494. XU, P., AND K. W. SPITZER. Na^+-independent Cl^--HCO_3^- exchange mediates recovery of pH_i from alkalosis in guinea pig ventricular myocytes. *Am. J. Physiol.* 267 (*Heart Circ. Physiol.* 36): H85—H91, 1994.
495. YAMADA, M., Y. HAMAMORI, H. AKITA, AND M. YOKOYAMA. P_2-purinoceptor activation stimulates phosphoinositide hydrolysis and inhibits accumulation of cAMP in cultured ventricular myocytes. *Circ. Res.* 70: 477–485, 1992.
496. YAMAWAKE, N., Y. HIRANO, T. SAWANOBORI, AND M. HIRAOKA. Arrhythmogenic effects of isoproterenol-activated Cl^- current in guinea-pig ventricular myocytes. *J. Mol. Cell. Cardiol.* 24: 1047–1058, 1992.
497. YAMAZAKI, J., F. BRITTON, M. L. COLLIER, B. HOROWITZ, AND J. R. HUME. Regulation of recombinant cardiac CFTR chloride channels by protein kinase C. *Biophys. J.* 76: 1972–1987, 1999.
498. YAMAZAKI, J., D. DUAN, R. JANIAK, K. KUENZLI, B. HOROWITZ, AND J. R. HUME. Functional and molecular expression of volume-regulated chloride channels in canine vascular smooth muscle cells. *J. Physiol. (Lond.)* 507: 729–736, 1998.
499. YAMAZAKI, J., AND J. R. HUME. Inhibitory effects of glibenclamide on cystic fibrosis transmembrane regulator, swelling-activated, and Ca^{2+}-activated Cl^- channels in mammalian cardiac myocytes. *Circ. Res.* 81: 101–109, 1997.
500. YANG, I. C., T.-H. CHENG, F. WANG, E. M. PRICE, AND T.-C. HWANG. Modulation of CFTR chloride channels by calyculin A and genistein. *Am. J. Physiol.* 272 (*Cell Physiol.* 41): C141—C155, 1997.
501. YANNOUKAKOS, D., A. STUART-TILLEY, H. A. FERNANDEZ, P. FEY, G. DUYK, AND S. L. ALPER. Molecular cloning, expression, and chromosomal localization of two isoforms of the AE3 anion exchanger from human heart. *Circ. Res.* 75: 603–614, 1994.
502. YURKO-MAURO, K. A., AND W. W. REENSTRA. Prostaglandin $F_{2\alpha}$ stimulates CFTR activity by PKA- and PKC-dependent phosphorylation. *Am. J. Physiol.* 275 (*Cell Physiol.* 44): C653—C660, 1998.
503. ZABEL, M., B. S. KOLLER, F. SACHS, AND M. R. FRANZ. Stretch-induced voltage changes in the isolated beating heart: importance of the timing of stretch and implications for stretch-activated ion channels. *Cardiovasc. Res.* 32: 120–130, 1996.
504. ZAKHAROV, S. I., AND R. D. HARVEY. Altered beta-adrenergic and muscarinic response of CFTR Cl^- current in dialyzed cardiac myocytes. *Am. J. Physiol.* 268 (*Heart Circ. Physiol.* 37): H1795—H1802, 1995.
505. ZAKHAROV, S. I., AND R. D. HARVEY. Rebound stimulation of the cAMP-regulated Cl^- current by acetylcholine in guinea-pig ventricular myocytes. *J. Physiol. (Lond.)* 499: 105–120, 1997.
506. ZAKHAROV, S. I., J. L. OVERHOLT, R. A. WAGNER, AND R. D. HARVEY. Tetramethylammonium activation of muscarinic receptors in cardiac ventricular myocytes. *Am. J. Physiol.* 264 (*Cell Physiol.* 33): C1625—C1630, 1993.
507. ZAKHAROV, S. I., S. PIERAMICI, G. K. KUMAR, N. R. PRABHAKAR, AND R. D. HARVEY. Nitric oxide synthase activity in guinea pig ventricular myocytes is not involved in muscarinic inhibition of cAMP-regulated ion channels. *Circ. Res.* 78: 925–935, 1996.
508. ZHANG, J., S. K. HALL, AND M. LIEBERMAN. An early transient current activates the swelling-induced chloride conductance in cardiac myocytes (Abstract). *Biophys. J.* 66: A442, 1994.
509. ZHANG, J., T. H. LARSEN, AND M. LIEBERMAN. F-actin modulates swelling-activated chloride current in cultured chick cardiac myocytes. *Am. J. Physiol.* 273 (*Cell Physiol.* 42): C1215—C1224, 1997.
510. ZHANG, J., AND M. LIEBERMAN. Chloride conductance is activated by membrane distention of cultured chick heart cells. *Cardiovasc. Res.* 32: 168–179, 1996.
511. ZHANG, J., R. L. RASMUSSON, S. K. HALL, AND M. LIEBERMAN. Hypo-osmotically activated Cl^- conductance in cultured chick heart cells (Abstract). *J. Gen. Physiol.* 100: 31a, 1992.
512. ZHANG, J., R. L. RASMUSSON, S. K. HALL, AND M. LIEBERMAN. A chloride current associated with swelling of cultured chick heart cells. *J. Physiol. (Lond.)* 472: 801–820, 1993.
513. ZHANG, K., P. L. BARRINGTON, R. L. MARTIN, AND E. R. TEN EICK. Protein kinase-dependent Cl^- currents in feline ventricular myocytes. *Circ. Res.* 75: 133–143, 1994.
514. ZHOU, S. S., A. TAKAI, M. TOMINAGA, AND Y. OKADA. Phosphatase-mediated enhancement of cardiac cAMP-activated Cl^- conductance by a Cl^- channel blocker, anthracene-9-carboxylate. *Circ. Res.* 81: 219–228, 1997.
515. ZOROV, D. B., K. W. KINNALLY, AND H. TEDESCHI. Voltage activation of heart inner mitochondrial membrane channels. *J. Bioenerg. Biomembr.* 24: 119–124, 1992.
516. ZYGMUNT, A. C. Intracellular calcium activates a chloride current in canine ventricular myocytes. *Am. J. Physiol.* 267 (*Heart Circ. Physiol.* 36): H1984—H1995, 1994.
517. ZYGMUNT, A. C., AND W. R. GIBBONS. Calcium-activated chloride current in rabbit ventricular myocytes. *Circ. Res.* 68: 424–437, 1991.
518. ZYGMUNT, A. C., AND W. R. GIBBONS. Properties of the calcium-activated chloride current in heart. *J. Gen. Physiol.* 99: 391–414, 1992.
519. ZYGMUNT, A. C., R. J. GOODROW, AND C. M. WEIGEL. I_{NaCa} and $I_{Cl(Ca)}$ contribute to isoproterenol-induced delayed afterdepolarizations in midmyocardial cells. *Am. J. Physiol.* 275 (*Heart Circ. Physiol.* 44): H1979—H1992, 1998.
520. ZYGMUNT, A. C., D. C. ROBITELLE, AND G. T. EDDLESTONE. I_{to1} dictates behavior of $I_{Cl(Ca)}$ during early repolarization of canine ventricle. *Am. J. Physiol.* 273 (*Heart Circ. Physiol.* 44): H1096—H1106, 1997.