Transient Outward Current, I_{to1}, Is Altered in Cardiac Memory

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Background—Cardiac memory refers to an altered T-wave morphology induced by ventricular pacing or arrhythmias that persist for variable intervals after resumption of sinus rhythm.

Methods and Results—We induced long-term cardiac memory (LTM) in conscious dogs by pacing the ventricles at 120 bpm for 3 weeks. ECGs were recorded daily for 1 hour, during which time pacing was discontinued. At terminal study, the heart was removed and the electrophysiology of left ventricular epicardial myocytes was investigated. Control (C) and LTM ECG did not differ, except for T-wave amplitude, which decreased from 0.12 ± 0.18 to -0.34 ± 0.21 mV ($\pm$SEM, $P<0.05$), and T-wave vector, which shifted from $-37\pm12°$ to $-143\pm4°$ ($P<0.05$). Epicardial action potentials revealed loss of the notch and lengthening of duration at 20 days (both $P<0.05$). Calcium-insensitive transient outward current (I_{to}) was investigated by whole-cell patch clamp. No difference in capacitance was seen in C and LTM myocytes. I_{to} activated on membrane depolarization to -25 ± 1 mV in C and -7 ± 1 mV ($P<0.05$) in LTM myocytes, indicating a positive voltage shift of activation. I_{to} density was reduced in LTM myocytes, and a decreased mRNA level for *Kv4.3* was observed. Recovery of I_{to} from inactivation was significantly prolonged: it was 531 ± 80 ms (n=10) in LTM and 27 ± 6 ms (n=9) in C ($P<0.05$) at -65 mV.

Conclusions—I_{to} changes are associated with and can provide at least a partial explanation for action-potential and T-wave changes occurring with LTM. (***Circulation*. 1999;99:1898-1905.**)

Key Words: electrocardiography ∎ ventricles ∎ pacing ∎ myocytes

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"Cardiac memory" refers to a change in the T wave induced by and persisting for minutes to months after cessation of a period of altered ventricular activation.[1–4] Canine studies have shown that short-term T-wave memory is prevented by intravenous 4-aminopyridine (4-AP), suggesting that the memory might depend on pacing-induced changes in the transient outward current, I_{to}.[5] Studies of an isolated tissue model of cardiac memory supported this view, demonstrating that an altered epicardial-endocardial voltage gradient contributes to memory and that 4-AP abolishes it.[6]

The expression of 4-AP–sensitive I_{to} is heterogeneous through the canine ventricular wall, with current density higher in epicardial than endocardial myocytes.[7,8] We hypothesized that the 4-AP–sensitive I_{to} might be altered during the evolution of cardiac memory and used a chronic canine model to study it. The persistence of memory long after cessation of pacing permitted us to perform our experiments without fear that decay of the memory phenomenon would occur. We focussed on the 4-AP–sensitive I_{to1} in normal canine epicardial myocytes and those from animals with

"long-term memory." For convenience, we use the term I_{to} instead of I_{to1} throughout.

Methods

Mongrel dogs weighing 20 to 26 kg premedicated with propafol 5 mg/kg IV were anesthetized with 3% isoflurane and subjected to a left thoracotomy under sterile conditions. A Medtronic 5069 screw-in pacemaker (contact diameter, 0.020 inches) was inserted transepicardially into the anteroapical left ventricle (LV) and attached to a Medtronic 8340 programmable pacemaker embedded in a subcutaneous pocket. Animals recovered for 2 to 3 weeks, during which time ECGs were recorded every 2 to 3 days with the animal resting comfortably in a sling. Within 3 weeks, all postsurgical ECG changes had disappeared, and stable, reproducible recording of standard limb leads was demonstrated over a period of 1 week. LV pacing was then initiated at 120 bpm and maintained for 23 hours of each day. Holter monitoring was performed for 2 to 3 days of the pacing period. In no animal was there <75% capture. While pacing was discontinued, the ECG was recorded continuously to permit determination of ST-T waves in sinus rhythm. After 20 to 22 days of pacing, we performed terminal studies, which began within 3 hours of discontinuation of ventricular drive.

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TABLE 1.   Effects of Chronic-Pacing on the ECG*

	Heart Rate, bpm	P-R Interval, ms	QRS Duration, ms	Q-T Interval, ms	Q-T$_c$ interval	T-Wave Amplitude, mV	T-Wave Vector, °
Control	93±15	106±8	58±2	234±3	285±22	0.12±0.18	−37±12
After pacing	83±6	111±9	56±3	231±8	271±16	−0.34±0.21†	−143±4†

*All data were recorded during sinus rhythm before and 1 to 4 hours after termination of long-term ventricular pacing at 20 to 22 days. Data are mean±SEM.

†$P<0.05$ vs control (paired t test).

Isolated Tissue and Single Myocyte Studies

Animals were anesthetized with pentobarbital sodium 30 mg/kg IV, and the heart was rapidly excised and placed in iced Tyrode's solution, containing (in mmol/L) NaCl 131, KCl 4, CaCl$_2$ 2.7, NaHCO$_3$ 18, MgCl$_2$ 0.5, NaH$_2$PO$_4$ 1.8, and dextrose 5.5, gassed with 95% O$_2$/5% CO$_2$. For microelectrode studies, strips of LV free wall epicardium obtained 1 cm below the mitral ring and measuring ≈1 cm×1 cm×0.5 to 1.5 mm were filleted from control dogs and those studied at 20 to 22 days of pacing and having cardiac memory. Tissues were placed in a chamber perfused with Tyrode's solution at 37±0.1°C and stimulated by standard techniques[6] via bipolar silver electrodes insulated with Teflon. The tissue bath was connected to ground with a 3 mol/L KCl-Ag-AgCl bridge. Glass microelectrodes filled with 3 mol/L KCl were used to impale the tissues. Standard means for calibration and recording were used.[6]

Single cells were isolated from control hearts and those with cardiac memory. The free-wall epicardial layer (1 to 3 mm) near the LV base, ≥2 cm from the pacemaker, was excised as above and subjected to trituration as reported previously.[9] Details of the transport solution and disaggregation have been described.[10] Disaggregated cells were kept in KB medium[11] at room temperature for ≥1 hour before electrophysiological experiments.

To avoid Ca^{2+} current contamination, I_{to} was recorded by the whole-cell patch-clamp technique in modified Tyrode's solution containing (in mmol/L) NaCl 137.7, NaOH 2.3, MgCl$_2$ 1, glucose 10, HEPES 5, KCl 5.4, CaCl$_2$ 1.8, MnCl$_2$ 2, and CdCl$_2$ 0.2, pH 7.4). Pipettes were filled with solution containing (in mmol/L) NaCl 6, potassium aspartate 130, MgCl$_2$ 2, CaCl$_2$ 5, EGTA 11, HEPES 10, Na$_2$-ATP 2, and Na-GTP 0.1; pH was adjusted to 7.2 by KOH. Pipette resistance was 2 to 4 MΩ. The pH of the Tyrode's solution containing 4-AP was titrated to 7.4 with HCl. Temperature was maintained at 30°C to 32°C. An Axopatch 1B amplifier (Axon Instruments, Inc) was used.

We used Tyrode's solution containing NaCl 137.7 mmol/L to facilitate comparison with action potential studies. Because I_{Na} was not blocked, we performed control experiments to determine whether the overlap of I_{Na} distorted our estimate of I_{to} threshold. No change in threshold occurred (ie, in 5 control myocytes, I_{to} threshold was −24±4 mV in both Na 140 and 10 mmol/L. At the same [Na$^+$]$_o$, the threshold in all 3 myocytes from memory animals was −10 mV.)

Data were collected by FM recording (Hewlett-Packard Co; 3964a, speed 7/8 in s-1, 600-Hz bandwidth) and pClamp software (Axon Instruments, Inc).

Preparation of RNA and RNase Protection Assay

Tissue samples were quick-frozen in liquid N$_2$ and homogenized in guanidinium thiocyanate. Total RNA was prepared by pelleting the homogenate over a CsCl step gradient. All RNA samples were quantified spectrophotometrically.

Canine *Kv4.3* and cyclophilin probes were prepared as previously.[12] Significant nonhybridizing sequence (≈50 bp) was included in the probes to facilitate distinction between the probe and the specific protected band. There was no evidence for unwanted cross-reaction between probes and nonspecific transcripts.

RNase protection assays were performed as previously.[13] For each sample point, 5 or 10 μg total RNA was used. A cyclophilin probe was included as an internal control to confirm that the sample was not lost or degraded during the assay. Yeast RNA 35 μg was a negative control to test for the presence of probe self-protection

bands. RNA expression was quantified directly from dried RNase protection gels by use of a PhosphorImager (Molecular Dynamics). There was no significant change in cyclophilin expression between the control and memory samples (average cyclophilin expression in long-term memory samples was 96% of control, $P>0.05$).

Statistical Analysis

Data are presented as mean±SEM. Comparisons of control and cardiac memory ECG from the same animals were done by paired t test. Comparisons of control and cardiac memory action potentials and ion currents were by t test for grouped data. A value of $P<0.05$ was considered significant.

Results

ECGs and Epicardial Action Potentials

Cardiac memory was seen as an altered T-wave vector angle and amplitude (Table 1). No other significant ECG changes occurred. Persistence of memory for 3 days after cessation of pacing is shown in Figure 1. Action potential characteristics for tissue slabs from control and memory epicardial muscle are presented in Table 2. The maximum diastolic potential and overshoot do not differ significantly in control and memory. However, the latter shows a smaller phase 1 notch and a longer action potential duration (APD) at day 20 (see example in Figure 2).

Capacitance of Myocytes

To test whether the pacing protocols might alter cell size, we determined the capacitance of disaggregated myocytes from control and memory epicardium. The capacitance of control myocytes was 104±8 pF (n=26) and that of memory myocytes, 107±9 pF (n=19) ($P>0.05$).

Properties of I_{to} in Normal and Memory Epicardial Myocytes

Activation Threshold

The action potential results suggested that alterations in I_{to} could be important to the genesis of long-term memory. Figure 3 demonstrates that I_{to} activates at a more positive potential in a myocyte from a dog with memory than in a control. The voltage thresholds for I_{to} in Figure 3 are −30 mV for the control and 0 mV for memory. The changes in I_{to} threshold are summarized in Table 3 and include an 18-mV positive shift in threshold. We could not construct complete activation curves because even at extremely positive voltages, saturation was not reached.

Inactivation

Voltage Dependence. We next examined steady-state dependence of inactivation on voltage. We held the cell at a given potential for 400 ms and then stepped to the same test
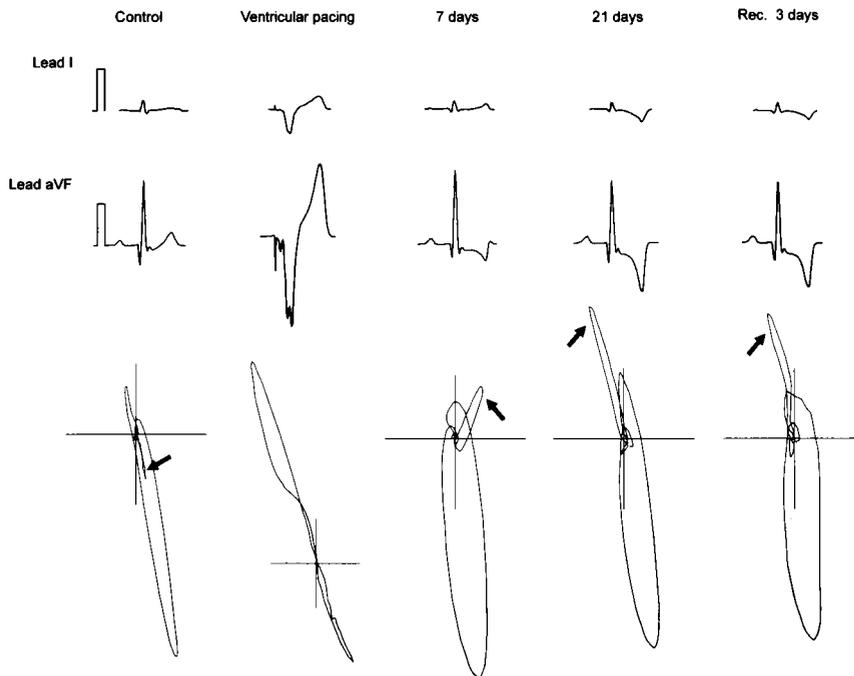
Figure 1. Top, Leads I and aVF of canine ECG in control (before ventricular pacing), during ventricular pacing, and 1 hour after discontinuing pacing on days 7 and 21 (last day of pacing), followed by recovery of T wave on day 3 after pacing was permanently discontinued (Rec). Bottom, Frontal plane vectorcardiogram from same animal. Note persistence of pacing day 21 memory T-wave change over 3 days after cessation of pacing. Paper speed, 25 mm/s; calibration, 1 mV. Arrow indicates T-wave vector during sinus rhythm.

potential of 0 mV (see protocol, Figure 4, inset). Results and a Boltzmann 2-state model fit to the data are provided in Figure 4, and midpoint values and slope factors are in Table 3. There is an 8-mV positive shift of the inactivation-versus-voltage curve in myocytes from hearts with memory, and no change in slope factor. Six additional experiments were performed with memory myocytes, with 2-second prepulses. No change in midpoint for inactivation voltage occurred (-35 ± 3 mV, n=6).

Kinetic Properties of Inactivation. Figure 5 shows results from a protocol demonstrating the inactivation kinetics of I_{to} from normal and memory myocytes. Each cell was held at -65 mV and depolarized to the values indicated on the figure. There were no significant differences in inactivation time constants, although a positive shift in their voltage dependence was observed (for average values for time constants of inactivation at $+20$ mV, see Table 3).

Despite the lack of significant difference in inactivation kinetics in memory, recovery from inactivation was dramatically altered (see Figure 6). From a holding potential of -65 mV, we depolarized to $+5$ mV in control and $+20$ mV in memory (to maintain approximately equal activation), returned to the holding potential for a variable time, and again depolarized to the test potential. The ratio of I_{to} amplitude in the second pulse compared with the amplitude in response to the first was plotted against time along with the best fit to a monoexponential function in Figure 6C. In Figure 6, the time constant of recovery was 33 ms in control and 531 ms in memory. As summarized in Table 3, the recovery time constant is more than an order of magnitude slower in cells from animals with memory.

Studies of hearts from several species have shown that I_{to} recovery from inactivation proceeds more rapidly with hyperpolarization.[14] We investigated whether a similar voltage dependence exists for control and memory myocytes (Figure 6D). Although I_{to} recovery from inactivation is an order of magnitude slower in memory than control, there is still the same speeding of recovery with hyperpolarization.

I_{to} Conductance

The changes in activation threshold and inactivation recovery kinetics provide a potential explanation for the reduction in the action potential notch recorded during cardiac memory. We also asked whether I_{to} conductance was reduced. Ideally, to estimate this conductance, one would saturate activation, measure tail currents at the same voltage, and normalize the tail currents to the capacitance of the cells. This was not possible because we could not fully activate the current at depolarized potentials. We therefore quantified I_{to} amplitude at each test potential and then divided current magnitude by the driving force to estimate the conductance at each poten-

TABLE 2. Epicardial Recordings From 2 Control Animals and Three 20-Day LV-Paced Animals

	n	MDP, $-$mV	Overshoot, mV	Phase I Notch, mV	APD_{50}, ms	APD_{90}, ms
Control	6	81 ± 1	14 ± 2	-1 ± 1	127 ± 6	184 ± 6
20-day paced	22	82 ± 1	11 ± 2	4 ± 2*	156 ± 6*	203 ± 5

MDP indicates maximum diastolic potential. Preparations were equilibrated for 3 hours at a basic cycle length of 650 ms.
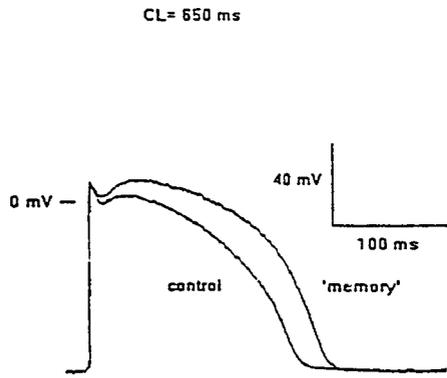
*$P < 0.05$ vs control.

CL= 650 ms

Figure 2. Representative experiments showing superimposed epicardial myocyte action potentials from 1 control dog and 1 dog paced for 20 days to induce memory. Note that although maximum diastolic potential is identical in both, in setting of memory, notch and plateau are more positive and APD is longer, basic cycle length (CL)=650 ms.

tial. We corrected the measured conductance by the difference in inactivation between control and memory myocytes that we measured in Figure 4 at the holding potential of −65 mV. Finally, we divided the measured values by the capacitance to obtain the normalized conductance against membrane potential (Figure 7). Because activation threshold differs in the 2 preparations, we could not compare conductance at the same voltage. Instead, we compared conductance by fitting the data by linear regression. The slope of the line gives the increase in conductance normalized to capacitance per change in membrane potential. This slope was 2.3 pS/(pF · mV) in control and 1.5 pS/(pF · mV) in memory. Thus, the slope of conductance versus potential for I_{to} in memory is ≈65% of control.
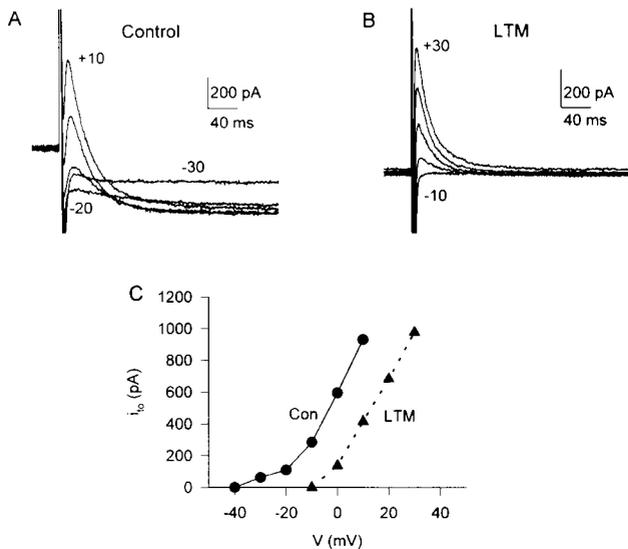

Figure 3. I_{to} in a normal (A) and a memory (LTM, B) ventricular myocyte. Currents were elicited by membrane depolarizing pulses of 400-ms duration from −30 to +10 mV with a 10-mV step in A and from −10 to +30 mV with a 10-mV step in B. Holding potentials in both were −65 mV. I_{to} amplitude was measured from its peak to steady-state level (end of pulses). I-V relationship was plotted in C for normal and memory. Holding current at −65 mV was +430 pA in A and +100 pA in B.

I_{to} Block by 4-AP

Figure 8 shows the effects of 4-AP on I_{to} in control (A, B, and C) and memory (D, E, and F). The holding potential was −65 mV for both cells, although the test potentials differed to allow for the differences in activation threshold. In both cells, 1 mmol/L 4-AP blocked ≈50% of the I_{to} at potentials ≥20 mV positive to voltage threshold. We found little difference in percentage block by 1 mmol/L 4-AP at +15 mV in control and memory (see Table 3).

Changes in *Kv4.3* mRNA

The *Kv4.3* gene encodes a potassium channel that underlies the bulk of I_{to} in canine ventricular myocytes.[12] In rat heart, regulation of I_{to} expression appears to be determined predominantly at the level of transcription.[13,15] Therefore, we were interested in ascertaining whether changes in the level of expression of the *Kv4.3* gene could contribute to the decline in I_{to} in memory. The value of *Kv4.3* expression in memory was 65±7% (n=6, $P<0.05$) of control (Figure 9), a change similar in magnitude to that in I_{to} conductance with voltage. This result suggests that expression of the *Kv4.3* gene is downregulated in ventricular muscle during memory and that this downregulation may contribute to changes in the I_{to} magnitude observed in the isolated cell recordings.

Discussion

Explanations accounting for cardiac memory have included an altered repolarization pattern resulting in a changed electrical gradient across the myocardium,[1,16] ischemia,[2,17,18] changes in mechanoelectrical feedback,[19] protein phosphorylation, and gene expression.[3] The hypothesis that ion channel alterations induce changes in the voltage-time course of repolarization and myocardial voltage gradients contributing to the evolution of memory is based on the following:

First, the T wave is generated by the voltage gradient during ventricular repolarization. Action potential configurations vary transmyocardially, one significant difference being the phase 1 "notch" seen in epicardium and midmyocardium but not endocardium.[14,20] I_{to} appears to be largely responsible for this notch[14,20]; when I_{to} is blocked, the plateau is elevated and the time course of repolarization changes.

Second, epicardial action potentials recorded during memory in isolated tissue models[6] and the present experiment lack a notch; they resemble those from endocardium. This observation led to the hypothesis that cardiac memory is largely due to a change in the voltage gradient that normally exists between epicardium and endocardium.[6] It was also hypothesized that if this voltage gradient were minimized by pharmacological interventions, memory might be prevented. This hypothesis gained support when 4-AP (an I_{to} blocker) prevented development of memory in the intact canine heart[5] and an isolated tissue model.[6]

The above-described work was performed in models of short-term cardiac memory, lasting minutes. Such models are of limited value in evaluating ion channel contributions to memory, because the electrophysiological changes do not persist long enough to ensure a stable substrate for myocardial disaggregation and whole-cell study. The use of a long-term memory model that we have demonstrated to be

TABLE 3. Properties of I_{to} in Control and Long-Term Memory Canine Epicardium

Preparation	Capacitance, pF	Activation, mV	Inactivation midpoint, mV	Slope factor, mV	τ_{Ina}, ms (+20 mV)	τ_{rec}, ms (−65 mV)	% Block (+15 mV) at 1 mmol/L 4-AP
Control	104±8 (n=26)	−25±1 (n=24)	−43±3 (n=12)	5±1 (n=12)	18±1 (n=14)	27±6 (n=9)	47±7 (n=6)
Long-term memory	107±9 (n=19)	−7±1 (n=31)*	−35±2* (n=9)	5±1 (n=9)	14±2 (n=8)	531±80 (n=10)*	64±6 (n=3)

τ_{Ina} indicates inactivation time constant; τ_{rec}, recovery time constant.

*$P<0.05$ vs control.

unassociated with cardiac failure[21] or hypertrophy (cell capacitance, reflecting cell size, is unchanged) provides long-term T-wave changes that render the study of disaggregated myocytes practicable. Moreover, we have previously shown that the statistically insignificant variations in sinus rate occurring before and after pacing cannot be the cause of these T-wave changes.[21]

The action potentials we recorded are entirely consistent with results from earlier isolated tissue studies in that the notch of the epicardial action potential in T-wave memory is smaller than in controls.[6] In short-term pacing, however, the epicardial APD did not manifest the prolongation seen with long-term pacing and cardiac memory in our present or previous study. The fact that the APD is not significantly prolonged in a memory model of short duration[6] suggests that in the long-term setting, repolarizing currents other than I_{to} may be involved (possibly $I_{Ca,L}$, I_K, Na/K pump current, I_{K1}). This possibility is also suggested by the study of Shvilkin et al.[21] Here, epicardial, endocardial, and midmyocardial action potentials were studied with microelectrodes in animals paced for ≥20 days. Significant prolongation of epicardial and endocardial action potentials as well as reduction of the difference in duration between the 2 types of action potentials occurred, such that epicardial action potentials prolonged to a greater extent than endocardial. In contrast, APDs of midmyocardial cells were unchanged. Hence, with memory, there was a reorientation of the interrelationships of APDs of each myocardial tissue. A prolongation of endocardial action potentials, which have a small notch and little I_{to}, and no change in duration of M cell action potentials, which have significant I_{to}, provides a further argument that currents other than I_{to} are involved in the memory process.

Kinetics of I_{to}

Despite the need to expand our studies to include other ion channels, our observations concerning I_{to} provide an understanding of the fundamental alterations in one ion channel that contribute to the memory phenomenon. Specifically, the shift in long-term memory I_{to} activation toward more positive potentials by almost +20 mV (Table 3) is consistent with the absence of a notch in ventricular epicardial action potentials from dogs with cardiac memory. I_{to} activation at relatively positive potentials will contribute reduced outward current to phase 1 repolarization. In the physiological voltage range, the memory-induced positive shift of I_{to} activation along the voltage axis in epicardium mimics the activation of I_{to} in endocardium and therefore would decrease the voltage gradient from epicardium to endocardium.

A significantly longer time course of I_{to} recovery from inactivation was found in memory myocytes. This phenomenon is an additional contributor to the loss of the action potential notch recorded in epicardial myocytes from animals with cardiac memory; eg, at a ventricular pacing rate 20% greater than sinus rate, the basic cycle length was ≈500 ms. Here, a 531-ms time constant of recovery from inactivation will not allow I_{to} to recover to a significant degree (even if it is activated at the end of phase 0).

A major question relates to the identity of the signaling mechanism responsible for the change in I_{to}. Our work in progress[22,23] and the published literature[3,19,24] suggest that altered stress-strain relationships induced by ventricular pacing activate the endogenous cardiac renin–angiotensin II system; this protein kinase C–linked signaling cascade both alters channel phosphorylation (perhaps explaining the changes in I_{to} in short-term memory) and induces the immediate early gene program, which alters new protein (channel) synthesis. Although we hypothesize that new gene expression and protein synthesis are involved in long-term memory, we do not know which genes are expressed and which new proteins are synthesized. Even though the sensitivity to 4-AP is not significantly changed in long-term memory myocytes,
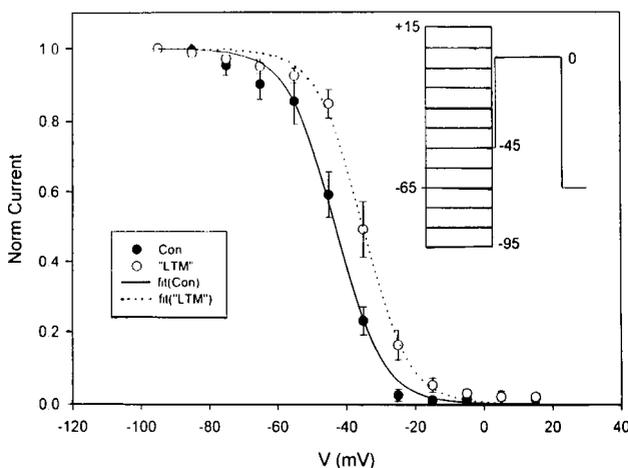
Figure 4. Steady-state voltage inactivation of I_{to} in normal (Con) and memory (LTM) myocytes. Inset shows protocol. Membrane was held at −65 mV and stepped to different prepulse potentials for 400 ms and then to −45 mV for 20 ms to avoid I_{Na} contamination, and finally to same test potential (0 mV). Currents were normalized (Norm) to maximum current and plotted against prepulse voltages. Averaged data were best fitted by a Boltzmann equation, with $V_{1/2}$=−43 mV and slope=5 mV (n=12) for control, and $V_{1/2}$=−35 mV and slope=5 mV (n=9) for long-term memory, respectively.
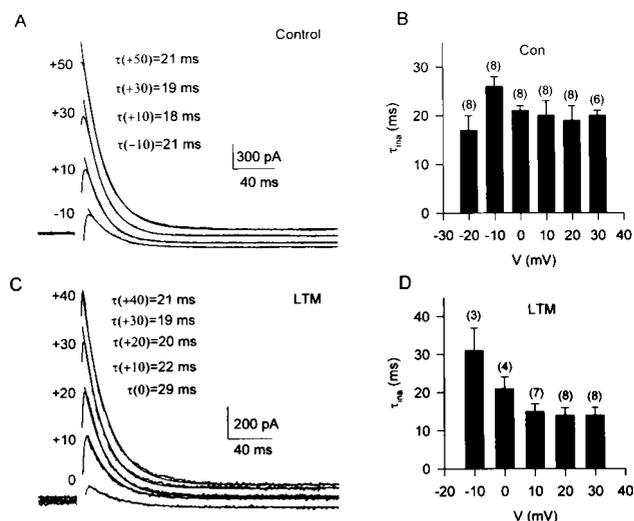
Figure 5. I_{to} inactivation kinetics in control and memory (LTM) myocytes. A, Four current traces in a control myocyte: each is well fit by 1 decaying exponential function; B, time constants of inactivation versus membrane potential for control; C, 5 current traces in a memory myocyte: each is well fit by 1 decaying exponential function; D, time constants of inactivation vs membrane potential for memory. Holding current=250 pA in A and 85 pA in B.

Figure 7. I_{to} density for control (Con) and memory (LTM) myocytes. Normalized conductance was calculated from normalized current density by dividing driving forces at various test membrane potentials, ie, G=I/ΔV (ΔV=E_{test}−E_K; E_K=−86 mV for potassium concentrations used). Given positive shift of I_{to} steady-state voltage inactivation in LTM (see Figure 4), I_{to} density in control myocytes was underestimated at holding potential, −65 mV, where normalized average value of I_{to} inactivation is 0.95 for LTM and 0.9 for control. To correct for this underestimate, control I_{to} density was multiplied by 0.95/0.9=1.0556. Data points were best fit by a linear function $y(x)=a_0+a_{1x}$, where a_1 indicates slope of straight line. Values are shown in inset.

this result does not completely rule out the possibility of an altered isoform, which remains to be investigated.

Clinical Importance

The clinical importance of cardiac memory initially was said to lie in its ECG patterns, mimicking those of ischemia.[1,4,17] However, the potential importance of the memory phenomenon appears to be greater, as follows: first, any event that alters repolarization might alter refractoriness as well, and changes in the effective refractory period can be permissive of or suppress arrhythmias. Second, in the setting of

tachycardia-induced CHF when heart rate is also rapid, abnormalities in I_{to} and in the T wave occur.[25] The possibility exists that the rate- and activation-induced changes in I_{to} induced by the memory phenomenon are important contributors to the T-wave anomalies in such pathological conditions. Third, recent studies by Allessie et al[26,27] have demonstrated pacing-induced atrial fibrillation in the goat. This, too, is associated with accelerated repolarization and loss of the action potential notch. Here, an atrial analog to the memory phenomenon might be considered. The statement by Wijffels
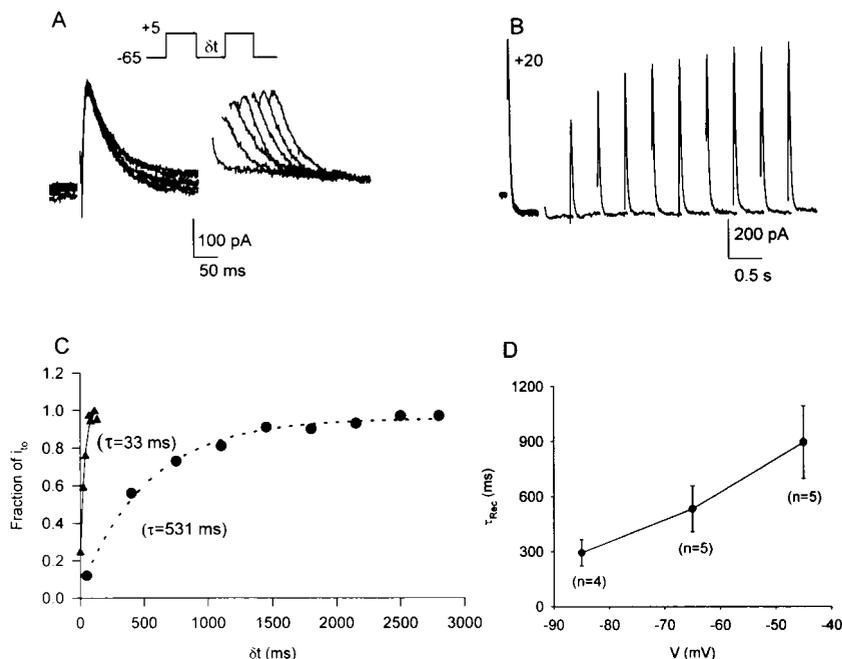
Figure 6. A, I_{to} recovery from inactivation in a control myocyte. A 2-pulse protocol as shown in inset of A was applied. Time constant of recovery (τ_{Rec}) was obtained by fitting data with 1 exponential function { y=a×[1−exp(−x/t)+b]}. B, I_{to} recovery from inactivation in a memory myocyte. C, Fraction of I_{to} with respect to first pulse is plotted against pulse interval. Data were fit by a single exponential. D, Voltage dependence of time constant for I_{to} recovery from inactivation in control (right ordinate) and memory (left ordinate). Holding current at −65 mV was +120 pA in A and +212 pA in B.
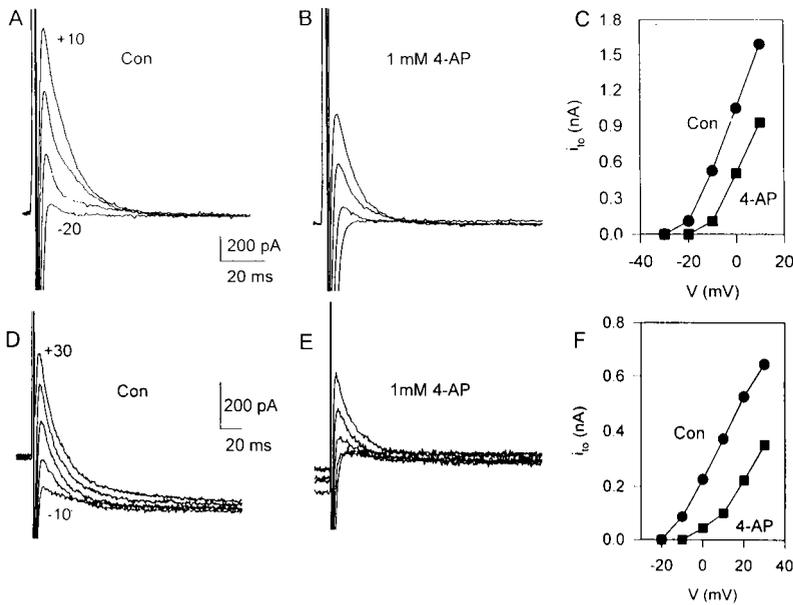
Figure 8. Inhibition of I_{to} by 1 mmol/L 4-AP in a control (A–C) myocyte. A, Depolarizing pulses were applied from −20 to +10 mV in 10 mV steps; B, 1 mmol/L 4-AP was added to bath solution; C, I-V relationship plotted for control I_{to} and in 1 mmol/L 4-AP. D–F, Inhibition of I_{to} by 1 mmol/L 4-AP in a memory myocyte; D, depolarizing pulses were applied from −10 to +30 mV in 10 mV steps; E, 1 mmol/L 4-AP was added to bath solution; F, I-V relationship for control I_{to} and effect of 1 mmol/L 4-AP on I_{to}. Holding current at −65 mV is +454 pA in A, +364 pA in B, +261 pA in D, and +128 pA in E.

et al that "fibrillation begets fibrillation"[27] leads us to suggest that any change in rhythm that alters activation pathway and rate might alter the action potential and render persistence/recurrence of the arrhythmia more likely. Similarly, maintenance of sinus rhythm and normal activation and/or interventions that maximize I_{to} might turn out to be antiarrhythmic. Finally, these initial results demonstrate that this stable canine model for cardiac memory can provide a "window" through which the alterations of ion channel characteristics that underlie altered repolarization and their relationship to signal transduction pathways can be investigated intensively.
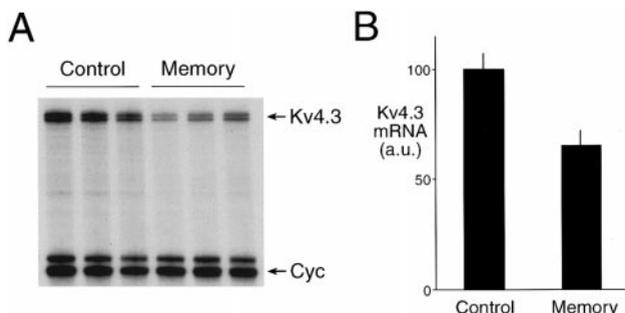
Acknowledgments

Figure 9. *Kv4.3* mRNA expression declines in cardiac memory. A, RNase protection analysis of *Kv4.3* expression in RNA samples from LV muscle of control and memory animals. Cyclophilin (Cyc) probe is internal control. B, Histogram of mean values for *Kv4.3* expression in control and memory animals expressed in arbitrary units (a.u.), normalized to a mean control value of 100%. Average *Kv4.3* mRNA expression in long-term memory is 65±7% of control ($P<0.05$, n=6).

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