

# Effect of acidosis on transient outward potassium current in isolated rat ventricular myocytes

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**Hulme, J. T., and C. H. Orchard** Effect of acidosis on transient outward potassium current in isolated rat ventricular myocytes. *Am. J. Physiol. Heart Circ. Physiol.* 278: H50–H59, 2000.—The effect of acidosis on the transient outward  $K^+$  current ( $I_{to}$ ) of rat ventricular myocytes has been investigated using the perforated patch-clamp technique. When the holding potential was  $-80$  mV, depolarizing pulses to potentials positive to  $-20$  mV activated  $I_{to}$  in subepicardial cells but activated little  $I_{to}$  in subendocardial cells. Exposure to an acid solution (pH 6.5) had no significant effect on  $I_{to}$  activated from this holding potential in either subepicardial or subendocardial cells. When the holding potential was  $-40$  mV, acidosis significantly increased  $I_{to}$  at potentials positive to  $-20$  mV in subepicardial cells but had little effect on  $I_{to}$  in subendocardial cells. The increase in  $I_{to}$  in subepicardial cells was inhibited by 10 mM 4-aminopyridine. In subepicardial cells, acidosis caused a  $+8.57$ -mV shift in the steady-state inactivation curve. It is concluded that in subepicardial rat ventricular myocytes acidosis increases the amplitude of  $I_{to}$  as a consequence of a depolarizing shift in the voltage dependence of inactivation.

heart; cardiac electrophysiology; subepicardium; subendocardium

THE TRANSIENT OUTWARD  $K^+$  current ( $I_{to}$ ) is present throughout the heart of many species; it is found in atrial and ventricular muscle, the sinoatrial node, atrioventricular node, and Purkinje fibers (see Ref. 8 for review). This current is an important determinant of the early repolarization phase of the cardiac action potential, and previous studies have shown a marked heterogeneity in action potential configuration within the ventricular wall that has been ascribed to regional differences in the density of  $I_{to}$ . Thus the action potential has a characteristic “spike and dome” morphology in the subepicardium, where  $I_{to}$  is prominent, that is less marked in the subendocardium, where  $I_{to}$  is small or absent (3, 21).

Given its important role in determining the configuration of the cardiac action potential, and the heterogeneity in the density of  $I_{to}$  in different regions of the heart, interventions that modulate  $I_{to}$  may have important consequences on action potential dispersion and hence on the spread of the action potential through the heart. This may be important in a number of pathologi-

cal conditions that have been reported to alter  $I_{to}$ , for example, metabolic inhibition (25) and heart failure (5). Antzelevitch et al. (3) reported that simulated ischemia caused a marked depression of the subepicardial action potential, which could be reversed by 4-aminopyridine (4-AP), an inhibitor of  $I_{to}$ , but had little effect on the subendocardial action potential. These data suggest that an increase in  $I_{to}$  may, at least in part, underlie the changes in action potential configuration recorded during simulated ischemia so that the regional differences in the response to such “ischemia” may reflect regional differences in the density of  $I_{to}$ .

Because acidosis is known to have marked effects on several membrane currents (for reviews see Refs. 23, 24) and is a major component of both the simulated ischemia described above and of true ischemia, then if acidosis increases  $I_{to}$ , this could explain the changes in action potential configuration observed by Antzelevitch et al. (3) and could play a role in altering the electrical activity of the heart during true ischemia. We have, therefore, investigated the effects of acidosis on  $I_{to}$  in cells isolated from the subepicardial and subendocardial regions of the rat left ventricle.

## MATERIALS AND METHODS

### Cell Isolation

Rat ventricular myocytes were isolated as described previously (13). Briefly, adult Wistar rats of either sex were stunned and then killed by cervical dislocation. The heart was rapidly removed and transferred to a beaker containing oxygenated physiological salt solution (PSS; for composition see below) where it was gently massaged to remove excess blood. The aorta was cannulated, and the heart was retrogradely perfused at a constant flow ( $8 \text{ ml} \cdot \text{min}^{-1} \cdot \text{g wet wt}^{-1}$ ) with PSS containing  $\text{Ca}^{2+}$  (0.75 mM) at  $37^\circ\text{C}$ .

Once the preparation appeared stable and the coronary vessels had cleared of blood, the perfusion was switched to nominally  $\text{Ca}^{2+}$ -free PSS for 4 min. The perfusate was then switched to PSS containing 1 mg/ml collagenase (type II, Worthington), 0.1 mg/ml protease (type XIV, Sigma), and  $50 \mu\text{M}$   $\text{Ca}^{2+}$ . This solution was recirculated to give a total exposure to the enzyme of 10 min.

At the end of the perfusion, the heart was cut down and the ventricles dissected free. The ventricles were then cut open, and thin slices of tissue were cut from the subepicardium ( $<2$  mm from the epicardial surface) and the subendocardium ( $<2$  mm from the endocardial surface) of the left ventricle using a fine pair of scissors. The slices were cut parallel to the surface of the ventricular wall and along the apex-base axis. The slices from each region were placed in separate conical flasks and gently agitated in enzyme-containing PSS supplemented

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with 1% BSA (Sigma) for 5-min periods at 37°C. Cells from each 5-min incubation were harvested by filtration followed by centrifugation at 400 rpm for 40 s. The supernatant was removed, and the cells were resuspended in PSS containing 0.75 mM  $\text{Ca}^{2+}$ . This procedure was repeated four or five times for tissue from each region, and the cells stored until use.

### Experimental Procedure

When required, a drop of cell suspension containing subepicardial or subendocardial cells was placed in a small chamber (volume 0.1 ml) on the stage of a Nikon Diaphot inverted microscope. After 10–15 min, when the cells had settled onto the glass bottom of the chamber, the chamber was perfused at ~2.5 ml/min with a HEPES-buffered PSS (HPS; for composition see below) at room temperature (22°C). Miniature solenoid valves (Lee Products) were used to direct one of up to four different solutions to the chamber. Solution was removed continuously to waste using suction.

### Perforated Patch Recording

Membrane current was recorded using the perforated patch-clamp technique. Briefly, glass pipettes were pulled from nonheparinized hematocrit tubes to a resistance of 2–4 M $\Omega$ . The tip of the pipette was first filled with an antibiotic-free intracellular solution (see below for composition) that contained 1 mM  $\text{Ca}^{2+}$ , to detect accidental rupture of the patch membrane (i.e., whole cell configuration), in which case the cell died rapidly; the pipette was then backfilled with the same solution plus 0.24 mM amphotericin B. After correction of the liquid junction potential and gigaohm seal formation, 20-ms voltage command pulses from –40 to 0 mV were applied to the pipette using an Axopatch ID (Axon Instruments) patch-clamp amplifier. Electrical access was usually obtained within 5–10 min, and 20–30 min were allowed before starting the experiment. Pipette capacitance was electronically compensated, and membrane current was filtered at 1.5 kHz (low-pass Bessel filter). Signals were displayed on an oscilloscope (Tektronix 5111A) and pen recorder (Gould, RS3600). The signals were also recorded onto videotape via a pulse-code modulator (Neuro-Corder, Neuro Data Instruments, and JVC HR-D367 video recorder) and computer via a CED 1401 analog-to-digital converter (Cambridge Electronic Design) for later off-line analysis.

### Solutions and Drugs

The PSS used during the cell isolation contained (in mM) 130 NaCl, 5.4 KCl, 0.4  $\text{Na}_2\text{PO}_4$ , 1.4  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ , 10 HEPES, 10 glucose, 20 taurine, 10 creatine, 0.75  $\text{Ca}^{2+}$ , with pH set to 7.3 using 2 M NaOH. The enzyme-containing solution consisted of isolation solution plus 1 mg/ml collagenase (type I, Worthington), 0.1 mg/ml protease (type XIV, Sigma), and 50  $\mu\text{M}$   $\text{CaCl}_2$ . The HPS used during the experiments contained (in mM) 130 NaCl, 5 KCl, 1  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ , 1  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 20 sodium acetate, 10 glucose, 5 HEPES, 1  $\text{CaCl}_2$ , and 5 U/l insulin, pH 7.4. The pipette solution contained (in mM) 110 potassium glutamate, 10 KCl, 10 NaCl, 1  $\text{MgCl}_2$ , 5 HEPES, and 1  $\text{CaCl}_2$ , pH 7.1.

Extracellular acidosis was produced by lowering the pH of the HPS bathing solution from 7.4 to 6.5. Reducing extracellular pH ( $\text{pH}_o$ ) in a HPS reduces intracellular pH ( $\text{pH}_i$ ) by 20–40% of the change of  $\text{pH}_o$  in ventricular muscle (6). Thus, in the present study, we would expect  $\text{pH}_i$  to decrease from its normal value of ~7.1 to between pH 6.9 and 6.7 in the presence of the acid solution. Previous work in our laboratory

(18) has shown that  $\text{pH}_i$  decreases to a new steady state within 5-min exposure to this type of acidosis so that all of the measurements reported in the present paper were obtained after at least 5-min exposure to the acid solution. Nifedipine (10  $\mu\text{M}$ ) was always present in the bathing solution to block the L-type calcium current ( $I_{Ca}$ ). 4-AP was prepared as a 100 mM stock solution (pH set to 7.4 using 1 M HCl) that was diluted to give a final concentration of 10 mM. Care was taken to adjust the pH of the experimental solution to the desired value (pH 7.4 or 6.5) after the addition of 4-AP. 1,2-Bis(2-aminophenoxy)ethane- $N,N,N',N'$ -tetraacetic acid (BAPTA)-AM was dissolved in DMSO to produce a 1 M stock solution that was added to the bathing solution to give a final concentration of 5  $\mu\text{M}$ . This concentration of BAPTA-AM was sufficient to abolish contraction in field-stimulated myocytes in the absence of nifedipine (data not shown). We have previously shown (18) that BAPTA does not affect  $\text{pH}_i$  or its response to acidosis.

### Experimental Protocol and Data Analysis

The holding potential was set to either –80 or –40 mV, as described in RESULTS, and voltage-clamp pulses were applied at 0.5 Hz. When the holding potential was –80 mV, 50-ms depolarizing pulses to –40 mV were applied (to inactivate the  $\text{Na}^+$  current) immediately before the 200-ms depolarizing test pulses to various potentials that were used to activate  $I_{to}$ . The magnitude of the transient outward component of  $I_{to}$  during each test pulse was measured as the difference between peak outward current and the current remaining at the end of the 200-ms depolarizing pulse. The magnitude of the sustained outward current was measured as the difference between the current remaining at the end of the 200-ms depolarizing pulse and the holding current between pulses. Acidosis-sensitive, 4-AP-sensitive, and BAPTA-sensitive currents are presented as the difference between the currents measured in the presence of acidosis, 4-AP, or BAPTA and those measured in their absence.

All data are expressed as means  $\pm$  SE of  $n$  cells. Statistical comparisons were made using an unpaired or paired  $t$ -test as appropriate. Values of  $P < 0.05$  were taken to indicate statistical significance.

## RESULTS

### Effect of Acidosis on $I_{to}$ in Different Regions of the Rat Left Ventricle

Figure 1 shows superimposed records of  $I_{to}$  in a representative subepicardial and subendocardial cell, recorded from a holding potential of –80 mV at control pH ( $\text{pH}_o$  7.4; Fig. 1A) and after 5-min exposure to the acid solution ( $\text{pH}_o$  6.5; Fig. 1B). At control pH, 200-ms depolarizing pulses to potentials more positive than –20 mV resulted in the voltage-dependent activation of  $I_{to}$  in cells isolated from the subepicardium (Fig. 1A, left). In contrast, little  $I_{to}$  was recorded in cells isolated from the subendocardium (Fig. 1A, right). In eight subepicardial cells, mean current amplitude at +60 mV (measured as described in MATERIALS AND METHODS) was  $1.79 \pm 0.35$  nA, whereas current amplitude at +60 mV in four subendocardial cells was  $0.14 \pm 0.09$  nA ( $P < 0.001$ ). Currents recorded from the same subepicardial and subendocardial cells during acidosis are shown in Fig. 1B. Five-minute exposure to the acid solution did not significantly alter  $I_{to}$  in either subepicardial or

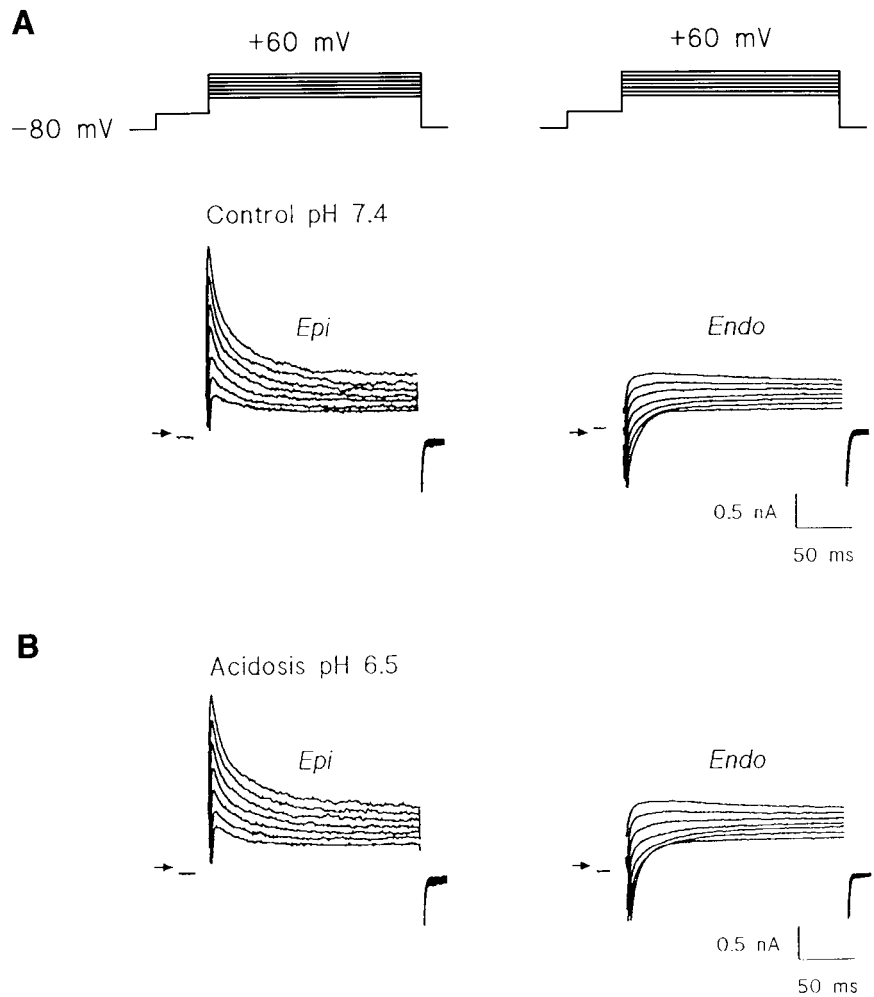


Fig. 1. Effect of acidosis on transient outward  $K^+$  current ( $I_{to}$ ) in a subepicardial cell and subendocardial cell from a holding potential of  $-80$  mV. *A* and *B*: voltage-clamp pulse protocol (*top*) and superimposed records of membrane current recorded from a holding potential of  $-80$  mV at control pH [extracellular pH ( $pH_o$ ) 7.4; *A*] and during acidosis ( $pH_o$  6.5; *B*) in a representative subepicardial (Epi; *left*) and subendocardial cell (Endo; *right*). Membrane current was recorded during 200-ms depolarizing pulses to potentials between 0 and  $+60$  mV. Arrows indicate the zero-current level, and the gaps in the current records indicate where current during prepulse to  $-40$  mV (see MATERIALS AND METHODS) has been removed for clarity.

subendocardial cells; mean current amplitude during acidosis at  $+60$  mV in subepicardial and subendocardial cells was  $1.81 \pm 0.33$  nA ( $n = 8$ ) and  $0.15 \pm 0.07$  nA ( $n = 4$ ), respectively.

Figure 2 shows superimposed current records from a representative subepicardial and subendocardial cell, recorded from a holding potential of  $-40$  mV at  $pH_o$  7.4 (*A*) and 6.5 (*B*). At control pH, depolarizing pulses to potentials more positive than  $-20$  mV activated little  $I_{to}$  in cells from either the subepicardium (Fig. 2*A*, *left*) or subendocardium (Fig. 2*A*, *right*); mean current amplitude at  $+60$  mV in subepicardial and subendocardial cells was  $0.13 \pm 0.06$  nA ( $n = 8$ ) and  $0.12 \pm 0.05$  nA ( $n = 4$ ), respectively ( $P = NS$ ). Membrane currents recorded from  $-40$  mV in the same cells during acidosis are shown in Fig. 2*B*. After 5-min exposure to acidosis, depolarizing pulses to potentials positive to  $-20$  mV resulted in the voltage-dependent activation of an outward current in subepicardial cells (Fig. 2*B*, *left*); peak outward current amplitude at  $+60$  mV increased significantly ( $n = 8$ ,  $P < 0.001$ ) to  $1.28 \pm 0.12$  nA, and the current remaining at the end of the depolarizing pulse increased from  $0.35 \pm 0.04$  nA at  $+60$  mV at control pH to  $0.43 \pm 0.05$  nA during acidosis ( $n = 8$ ;  $P < 0.05$ ). In contrast, in subendocardial cells (Fig. 2*B*,

*right*), outward current recorded during acidosis was not significantly different from that at control pH (Fig. 2*A*, *right*;  $n = 4$ ). Acidosis-sensitive currents (obtained by subtracting membrane currents recorded at control pH from those obtained during acidosis) in a representative subepicardial and subendocardial cell are shown in Fig. 2*C*. In subepicardial cells (Fig. 2*C*, *left*), the acidosis-sensitive currents were outward currents that increased in amplitude as the voltage was stepped to more positive potentials and showed rapid activation and inactivation. In contrast, little acidosis-induced current was observed in subendocardial cells (Fig. 2*C*, *right*).

Figure 3 shows mean ( $\pm SE$ ,  $n = 8$ ) data comparing the effects of acidosis on the current-voltage relationship of subepicardial cells at the two holding potentials. Current-voltage relationships were constructed by plotting current amplitude (measured as described in MATERIALS AND METHODS) against test potential. Figure 3*A* shows that  $I_{to}$  activated from a holding potential of  $-80$  mV was large and not significantly altered by acidosis. However, at a holding potential of  $-40$  mV (Fig. 3*B*),  $I_{to}$  was small at control pH, but acidosis significantly ( $P < 0.001$ ) increased outward current at potentials more positive than  $-20$  mV. This is in contrast to the data

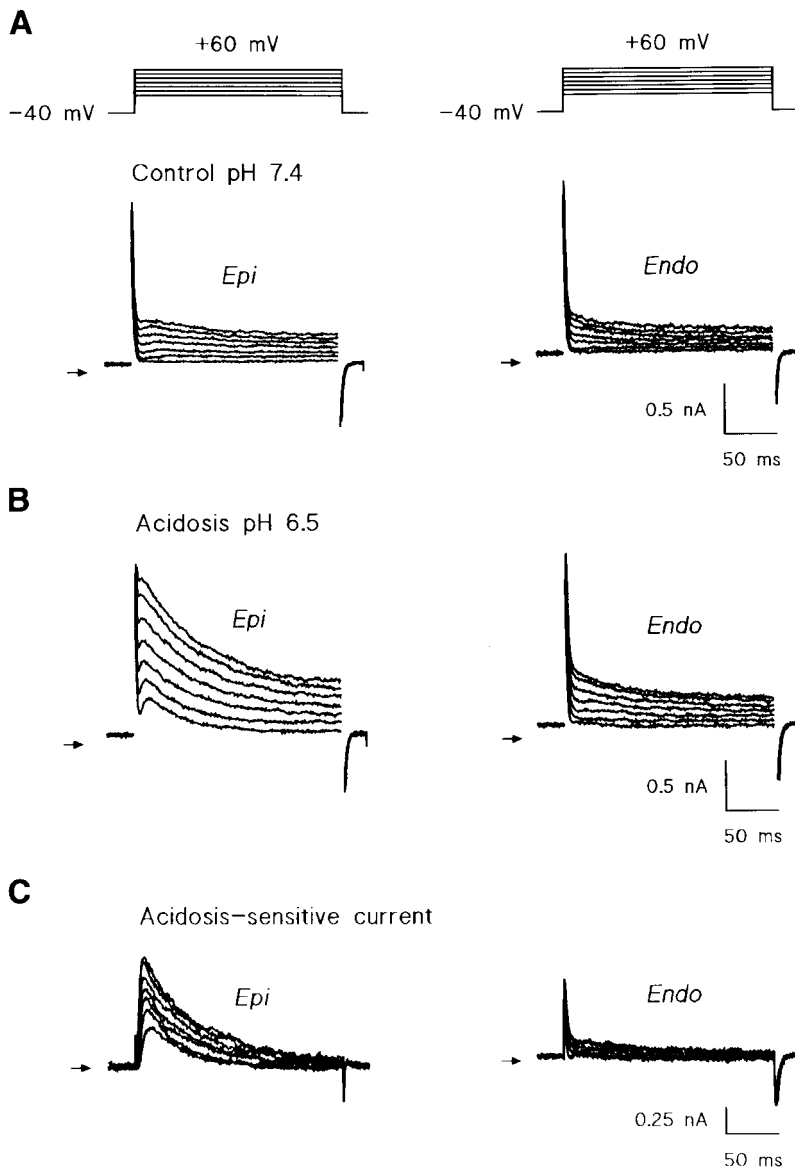


Fig. 2. Effect of acidosis on  $I_{to}$  in a subepicardial cell and a subendocardial cell from a holding potential of  $-40$  mV. *A* and *B*: membrane potential (top) and superimposed records of membrane current recorded from a holding potential of  $-40$  mV at control pH ( $\text{pH}_o$  7.4; *A*) and during acidosis ( $\text{pH}_o$  6.5; *B*) in a representative subepicardial (left) and subendocardial cell (right). Membrane current was recorded during 200-ms depolarizing pulses to potentials between 0 and  $+60$  mV. *C*: superimposed acidosis-sensitive current in subepicardial cells (left) and subendocardial cells (right), obtained by subtracting current at control pH from the current during acidosis. Arrows indicate zero-current level.

obtained in subendocardial cells, in which  $I_{to}$  was small at both holding potentials and was not significantly increased by acidosis.

#### Identity of the Acidosis-Sensitive Outward Current in Subepicardial Cells

To determine whether the acidosis-sensitive outward current recorded in subepicardial cells was  $I_{to}$ , the effect of 10 mM 4-AP, an inhibitor of  $I_{to}$ , was investigated. All effects of 4-AP shown were obtained in subepicardial cells, when the holding potential was  $-40$  mV, and were recorded after 3-min exposure to 4-AP. Figure 4 shows the effect of 10 mM 4-AP on membrane currents obtained in response to 200-ms depolarizing pulses to different test potentials during acidosis. During acidosis in the absence of 4-AP, depolarizing pulses to potentials positive to  $-20$  mV resulted in the rapid voltage-dependent activation of an outward current, which declined during the 200-ms pulse (Fig. 4A), as

described above. Application of 4-AP markedly reduced peak outward current, with little effect on the sustained component of membrane current (Fig. 4B). 4-AP-sensitive currents, obtained by subtracting current in the presence of 4-AP (Fig. 4B) from that in its absence (Fig. 4A), are shown in Fig. 4C. The amplitude of the 4-AP-sensitive currents was voltage dependent; these currents also showed rapid activation and inactivation, with almost complete decay by the end of the 200-ms depolarizing pulse. Figure 4D shows mean ( $\pm$ SE) current-voltage relationships at control pH, during acidosis, and during acidosis in the presence of 4-AP, showing that 4-AP significantly inhibited the acidosis-induced outward current amplitude at potentials positive to  $-20$  mV ( $n = 5$ ;  $P < 0.01$ ) so that the current-voltage relationship in the presence of 4-AP was not significantly different from that observed at control pH. These effects of 4-AP were reversed when 4-AP was removed from the perfusate (data not shown).

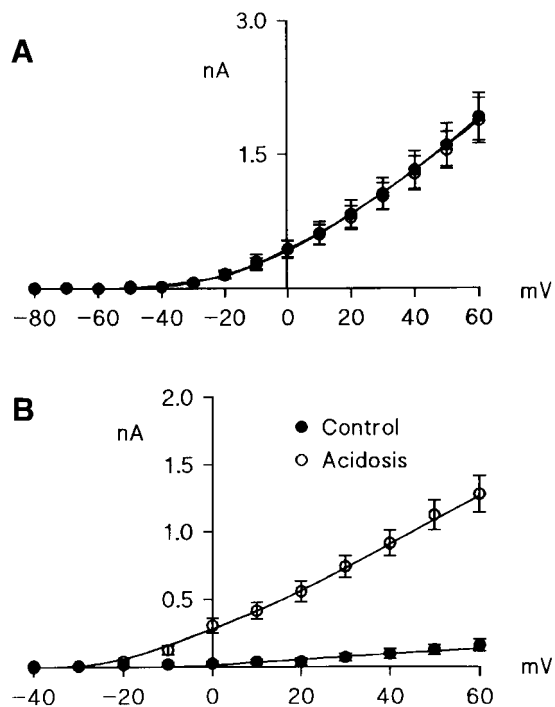


Fig. 3. Effect of acidosis on current-voltage relationship of  $I_{to}$  at holding potentials of  $-80$  and  $-40$  mV in subepicardial cells. *A* and *B*: current-voltage relationship of  $I_{to}$  at control pH<sub>o</sub> (●) and during acidosis (○) at holding potentials of  $-80$  mV (*A*) and  $-40$  mV (*B*) in subepicardial cells.  $I_{to}$  (measured as difference between peak outward current and current at the end of the 200-ms depolarizing pulse) is plotted against test potential. Means  $\pm$  SE are shown ( $n = 8$ ).

These data suggest, therefore, that the outward current activated by acidosis is  $I_{to}$  (see DISCUSSION).

#### Mechanisms Underlying the Acidosis-Induced Outward Current in Subepicardial Cells

**Voltage dependence of inactivation of  $I_{to}$ .** One possible explanation for the observed effect of acidosis on  $I_{to}$  is that the inactivation of  $I_{to}$  is shifted to more positive potentials during acidosis. To test this possibility, the effect of acidosis on the voltage dependence of inactivation of  $I_{to}$  was investigated using a double-pulse protocol; a 500-ms conditioning prepulse from a holding potential of  $-80$  mV to potentials between  $-80$  and  $0$  mV (in 10-mV steps) was followed by a 160-ms depolarizing test pulse to  $+60$  mV (Fig. 5).  $I_{to}$  was measured during the test pulse, and its magnitude was related to the potential during the conditioning pulse. Figure 5 shows superimposed current records obtained during the conditioning and test pulses at control pH (Fig. 5*A*) and during acidosis (Fig. 5*B*). Figure 5*A* shows that at control pH little inactivation of  $I_{to}$  during the test pulse was observed when the conditioning pulse potential was more negative than  $-60$  mV, but that the magnitude of  $I_{to}$  during the test pulse decreased (i.e., inactivation increased) as the conditioning pulse potential became more positive. Acidosis had no significant effect on the magnitude of  $I_{to}$  during the test pulse when the conditioning prepulse was more negative than  $-60$  mV but caused a shift in the voltage dependence of inactivation so that more  $I_{to}$  remained after a conditioning

prepulse to, e.g.,  $-40$  mV (see Fig. 5*B*). This is shown more clearly in Fig. 5*C*, which shows the effect of acidosis on the mean ( $\pm$ SE) steady-state inactivation curve of  $I_{to}$ .  $I_{to}$  during each test pulse was normalized to maximal  $I_{to}$  ( $I/I_{max}$ ) and plotted against the voltage during the conditioning prepulse. These data were fitted by the Boltzmann equation

$$I/I_{max} = 1/[1 + \exp[(V_{0.5} - V_m)/k]]$$

where  $V_{0.5}$  is the conditioning potential that gives  $I/I_{max} = 0.5$ ,  $V_m$  is the conditioning potential, and  $k$  describes the steepness of the curve.

Figure 5*C* shows that at control pH there was little inactivation of  $I_{to}$  during the test pulse when the conditioning prepulses were more negative than  $-60$  mV, but inactivation increased as the conditioning prepulse became more positive, and  $I_{to}$  was almost fully inactivated by conditioning prepulses positive to  $-30$  mV. The values of  $V_{0.5}$  and  $k$  at pH<sub>o</sub> 7.4 were  $-45.97$  mV and  $3.98$  mV, respectively ( $n = 8$ ). Figure 5*C* also shows that acidosis significantly ( $P < 0.05$ ) shifted the inactivation curve to more positive potentials (with no significant effect on the slope of the relationship) so that after conditioning prepulses to potentials between  $-50$  and  $-30$  mV, there was less inactivation during acidosis than at control pH. Acidosis caused an 8.57-mV depolarizing shift in the inactivation curve;  $V_{0.5}$  at pH<sub>o</sub> 6.5 was  $-37.4$  mV, and  $k$  was  $3.87$  mV ( $n = 8$ ). These data are compatible, therefore, with the hypothesis that the effects of acidosis on the amplitude of  $I_{to}$  are due to its effects on the inactivation of  $I_{to}$  (see DISCUSSION).

**$Ca^{2+}$  dependence of  $I_{to}$ .** Because intracellular  $Ca^{2+}$  concentration ( $[Ca^{2+}]_i$ ) increases during acidosis (2), and may affect  $I_{to}$  (10, 26), the possible role of  $[Ca^{2+}]_i$  in the observed response of  $I_{to}$  to acidosis was investigated using the  $Ca^{2+}$  chelator BAPTA-AM to buffer  $[Ca^{2+}]_i$ . Figure 6 shows superimposed records of  $I_{to}$  during acidosis before (*A*) and after (*B*) 10-min exposure to  $5 \mu M$  BAPTA-AM. Membrane currents were recorded during 200-ms depolarizing pulses to test potentials between  $0$  and  $+60$  mV from a holding potential of  $-40$  mV. The amplitude of  $I_{to}$  evoked from a holding potential of  $-40$  mV during acidosis was not significantly altered by buffering  $[Ca^{2+}]_i$ .  $I_{to}$  at  $+60$  mV before and after application of BAPTA was  $1.18 \pm 0.17$  and  $1.22 \pm 0.13$  nA, respectively ( $n = 4$ ). This suggests that an increase in cytoplasmic  $[Ca^{2+}]_i$  does not underlie the increase in amplitude of  $I_{to}$  that occurs during acidosis.

Figure 6 also shows that the time course of inactivation was more rapid in the presence of BAPTA. The decay of  $I_{to}$  at  $+60$  mV was fitted using a double exponential function that gave time constants ( $\tau$ ) for the fast phase of inactivation during acidosis of  $36.3 \pm 2.6$  ms before, and  $23.7 \pm 1.6$  ms after, the application of BAPTA-AM ( $P < 0.05$ ;  $n = 4$ ). However, the time constants for the slow phase of inactivation during acidosis before and after application of BAPTA-AM were not significantly different ( $1,523 \pm 387$  and  $1,160 \pm 213$  ms, respectively). In addition, BAPTA reduced the current remaining at the end of the depolarizing pulse

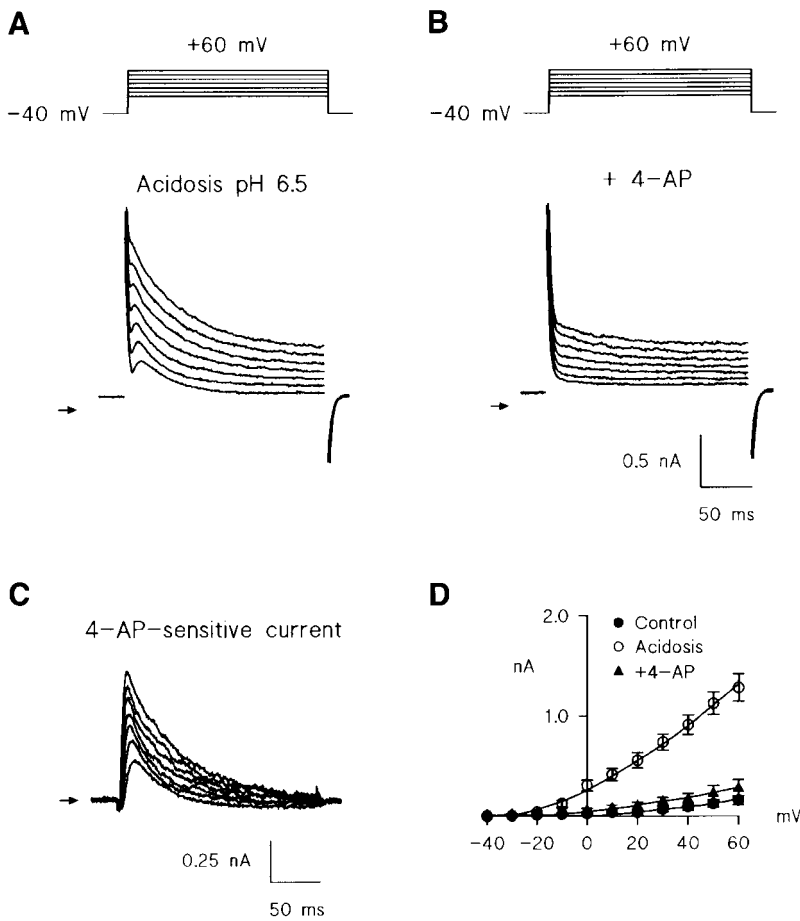


Fig. 4. Effect of 4-aminopyridine (4-AP) on acidosis-induced increase in  $I_{to}$  in subepicardial cells. *A* and *B*: superimposed records of membrane potential (*top*) and membrane current (*bottom*) recorded from a holding potential of  $-40$  mV during acidosis before (*A*) and after (*B*) application of 10 mM 4-AP. Membrane current was recorded during 200-ms depolarizing pulses to potentials between 0 and  $+60$  mV. *C*: superimposed 4-AP-sensitive currents, calculated by subtracting current after application of 4-AP from that before application of 4-AP during acidosis. Arrows indicate zero-current level. *D*: mean ( $\pm$ SE) current-voltage relationship of  $I_{to}$  at control pH ( $\bullet$ ) and during acidosis before ( $\circ$ ) and after ( $\blacktriangle$ ) application of 4-AP.

(Fig. 6) from  $0.38 \pm 0.07$  nA in the absence of BAPTA to  $0.27 \pm 0.08$  nA in the presence of BAPTA ( $n = 4$ ,  $P < 0.05$ ). Figure 6*C* shows the BAPTA-sensitive currents, which were obtained by subtracting the currents obtained during acidosis in the presence of BAPTA from those obtained in the presence of acidosis alone; these are sustained outward currents that show rapid activation on depolarization and decay slowly to an apparent plateau.

## DISCUSSION

### Identity of the Acidosis-Sensitive Outward Current in Subepicardial Cells

When the holding potential was  $-40$  mV, acidosis activated a rapidly activating and inactivating outward current in subepicardial cells. It appears likely that this current was  $I_{to}$  for several reasons: 1) it was blocked when  $K^+$  in the pipette solution was replaced by  $Cs^+$  (data not shown), suggesting that this current is carried predominantly by  $K^+$ ; 2) it was inhibited by 4-AP, an inhibitor of the  $Ca^{2+}$ -independent component of  $I_{to}$ ; 3) the 4-AP-sensitive and the acidosis-sensitive currents were similar, both exhibiting an amplitude, time course, and current-voltage relation characteristic of  $I_{to}$ ; and 4) the observation that the effects of acidosis were greater in subepicardial cells than subendocardial cells is consistent with the previously reported distribution of  $I_{to}$ . These data suggest that the outward current

activated by acidosis from depolarized potentials is  $I_{to}$ , consistent with a recent study by Stengl et al. (27).

However, because acidosis increases cytoplasmic  $[Ca^{2+}]$  (2) and alters  $Na^+/Ca^{2+}$  exchange activity, another possibility is that changes in  $Na^+/Ca^{2+}$  exchange current ( $I_{Na/Ca}$ ) might contribute to the observed current. However, nifedipine, which was present in the bathing solution to inhibit  $I_{Ca}$ , also abolishes the  $Ca^{2+}$  transient and contraction so that there should be little contamination of  $I_{to}$  by  $Ca^{2+}$  transient-induced activation of  $I_{Na/Ca}$ . The idea that the amplitude of  $I_{to}$  was not contaminated by  $I_{Na/Ca}$  is supported by the observation that BAPTA had little effect on the amplitude of  $I_{to}$  during acidosis (Fig. 6). Thus it seems most likely that the outward current activated by acidosis from depolarized holding potentials is  $I_{to}$ .

### Mechanism Underlying the Effect of Acidosis on $I_{to}$ in Subepicardial Cells

Although acidosis increased  $I_{to}$  in subepicardial cells, the effect was strongly voltage dependent; acidosis increased  $I_{to}$  when the holding potential was  $-40$  mV but had little effect on  $I_{to}$  when the holding potential was  $-80$  mV. This voltage dependence could be due to several possible mechanisms. One possibility is that acidosis alters the rate of recovery of  $I_{to}$  from inactivation. It has previously been shown (30) that recovery of  $I_{to}$  is voltage dependent, being slower at more depolar-

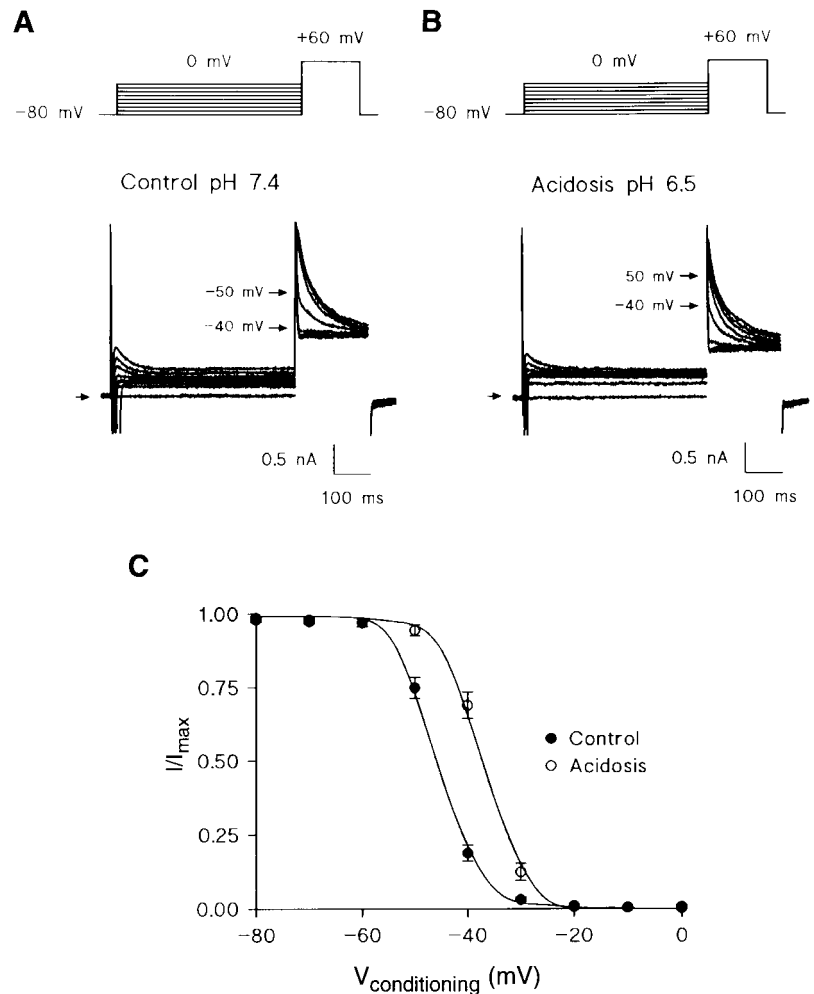


Fig. 5. Effect of acidosis on the steady-state inactivation of  $I_{to}$  in subepicardial cells. *A* and *B*: measurement of voltage dependence of inactivation. *Top panels* show protocol: 500-ms conditioning prepulses to various potentials (from  $-80$  to  $0$  mV in 10-mV increments) were followed by a 160-ms test pulse to  $+60$  mV. Holding potential was  $-80$  mV. *Bottom panels* show superimposed current records during conditioning pulses and subsequent test pulses to  $+60$  mV at control pH (*A*) and during acidosis (*B*). Arrows indicate zero-current level. *C*: mean ( $\pm$ SE;  $n = 8$ ) steady-state inactivation curves of  $I_{to}$ .  $I_{to}$  during each test pulse was normalized to maximal  $I_{to}$  ( $I/I_{max}$ ) and plotted against conditioning prepulse potential ( $V_{conditioning}$ ). Data are fitted with a Boltzmann equation. See text for further details.

ized potentials. However, even at  $-40$  mV, recovery is complete within 2 s (the interpulse interval used in the present study, Ref. 30). Thus if acidosis slowed recovery, it would be expected to decrease  $I_{to}$  rather than increase it as seen in the present study; if acidosis accelerated recovery, it would be expected to have little or no effect on the magnitude of  $I_{to}$ . It appears unlikely, therefore, that acidosis-induced changes in recovery can explain the present data. A second possibility is that the threshold for activation of  $I_{to}$  could be shifted to more negative potentials during acidosis, since protons may screen fixed negative charges on the membrane, thus altering the potential detected by the channel (16). However, this seems unlikely, because acidosis did not produce a leftward shift of the current-voltage relationship (Fig. 3).

A third possibility is that acidosis alters the voltage dependence of inactivation of  $I_{to}$ . In support of this hypothesis, acidosis caused a significant and reversible depolarizing shift in the steady-state inactivation of  $I_{to}$  (Fig. 5). At control pH in the present study, the half-maximal inactivation voltage and the slope of the inactivation curve were similar to those reported previously for  $I_{to}$  under similar experimental conditions (22), although more positive  $V_{0.5}$  values (by  $\sim 13$  mV) have also been reported (4, 9). This discrepancy may be

related to the experimental conditions used to isolate  $I_{to}$  from other currents. In particular, divalent cations, which are frequently used to block  $I_{Ca}$ , shift the activation and inactivation curves to more positive potentials (1).

At control pH, when the conditioning prepulse potential was  $-80$  mV, no inactivation of  $I_{to}$  was observed during the subsequent test pulse (Fig. 5). Because acidosis did not affect inactivation when the conditioning pulse was  $-80$  mV (i.e., there was still no inactivation; Fig. 5), this could explain why acidosis had no effect on  $I_{to}$  when the holding potential was  $-80$  mV (Fig. 3).

Changing the potential of the conditioning prepulse from  $-80$  to  $-40$  mV at control pH increased inactivation, thus reducing  $I_{to}$  during the subsequent test pulse by  $\sim 80\%$ , which could explain why changing the holding potential from  $-80$  to  $-40$  mV at control pH decreased  $I_{to}$ , measured at  $+60$  mV, by  $\sim 93\%$  (cf. Fig. 3).

Acidosis produced a depolarizing shift of the inactivation curve of  $\sim 8.6$  mV so that there was less inactivation after a conditioning prepulse to  $-40$  mV. Thus, when the holding potential was  $-40$  mV, significant inactivation of  $I_{to}$  would occur at control pH, but the acidosis-induced shift in the inactivation curve would decrease the proportion of inactivated current. After a

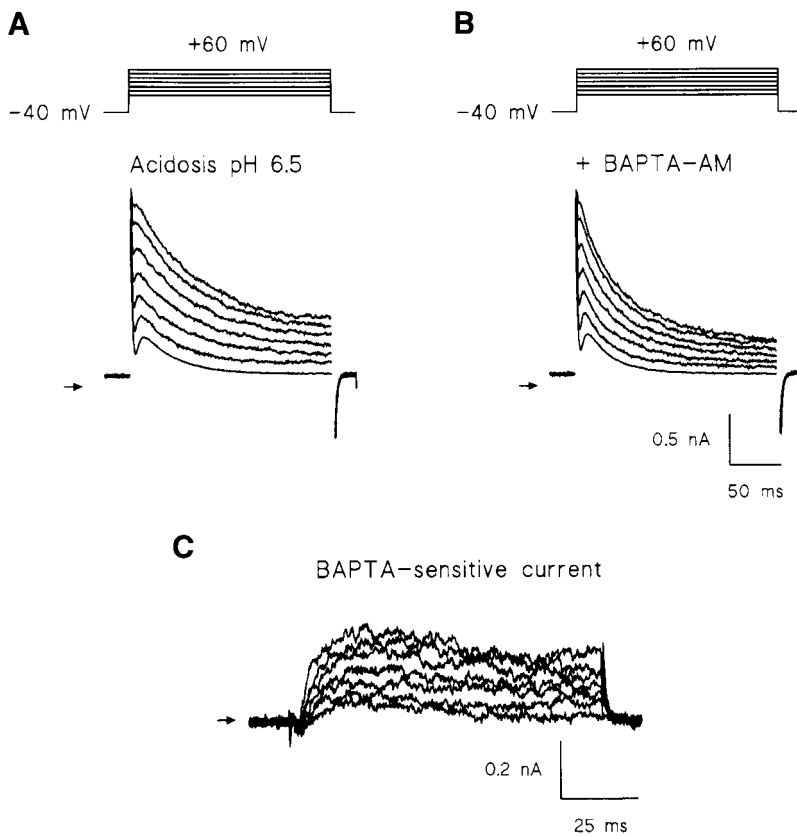


Fig. 6. Effect of buffering intracellular  $\text{Ca}^{2+}$  by BAPTA-AM on membrane current during acidosis in subepicardial cells at a holding potential of  $-40$  mV. *A* and *B*: superimposed records of membrane potential (top) and membrane current (bottom) recorded from same cell during acidosis before (*A*) and after (*B*) application of  $5 \mu\text{M}$  BAPTA-AM. Membrane current was recorded during 200-ms depolarizing pulses to potentials between 0 and  $+60$  mV (in 10-mV increments) from a holding potential of  $-40$  mV. *C*: superimposed records of BAPTA-sensitive currents calculated by subtracting currents after application of BAPTA-AM from those during acidosis alone. Arrows indicate zero-current level.

conditioning prepulse to  $-40$  mV during acidosis,  $I_{to}$  was  $\sim 75\%$  of maximal  $I_{to}$ , which could explain why acidosis increased  $I_{to}$ , measured at  $+60$  mV, to  $\sim 73\%$  of maximal  $I_{to}$  when the holding potential was  $-40$  mV (cf. Fig. 3). The rightward shift in the inactivation curve could, therefore, explain why acidosis increased  $I_{to}$  when the holding potential was  $-40$  mV.

The mechanism(s) underlying the shift in the voltage-dependent inactivation of  $I_{to}$  is unknown, but it appears likely that it involves specific effects on the channel protein rather than changes of membrane surface charge, because inactivation, but not activation, was altered by acidosis. It is not clear, however, whether acidosis is acting at an intracellular or extracellular site. During the present experiments, the observed change in  $I_{to}$  was almost complete within 45 s of exposure to the acid solution (data not shown). We have previously shown that within this time  $\text{pH}_i$  has decreased little (by  $\sim 25\%$  of its final change; Ref. 18), whereas with the bath volume (0.1 ml) and perfusion rate used (2.5 ml/min), the change of  $\text{pH}_o$  would be complete. Thus, although we cannot exclude an intracellular site of action, these data are compatible with an extracellular site being more important in the observed changes.

#### $\text{Ca}^{2+}$ Dependence of $I_{to}$

The existence of both  $\text{Ca}^{2+}$ -dependent and -independent components of  $I_{to}$  has been reported in several studies (10, 12, 14, 17, 28), whereas in others, the presence of a single  $\text{Ca}^{2+}$ -independent component of  $I_{to}$

has been described (4, 7, 11, 15, 19). In the present study, buffering  $[\text{Ca}^{2+}]_i$  with BAPTA had no effect on the amplitude of  $I_{to}$  (Fig. 6), suggesting that the increase in  $[\text{Ca}^{2+}]_i$  that occurs during acidosis does not underlie the increase in  $I_{to}$  observed in the present study. It is, however, worth noting that in the present study the  $\text{Ca}^{2+}$  transient was inhibited by nifedipine (so that  $I_{to}$  could be monitored, see MATERIALS AND METHODS) before the application of BAPTA-AM. Thus, in the present study, effects mediated by the  $\text{Ca}^{2+}$  transient would not be observed, and any changes observed (below) are likely to be due to the effects of acidosis on diastolic, rather than systolic,  $[\text{Ca}^{2+}]_i$ .

Although buffering  $[\text{Ca}^{2+}]_i$  during acidosis had no effect on the amplitude of  $I_{to}$ , inactivation was faster and the current remaining at the end of the pulse was decreased in the presence of BAPTA, suggesting that an acidosis-induced rise in  $[\text{Ca}^{2+}]_i$  might underlie, at least in part, the acidosis-induced increase in the sustained outward current. The amplitude, time course, voltage dependence, and 4-AP insensitivity of the BAPTA-sensitive currents recorded during acidosis (Fig. 6C) were similar to the sustained outward current reported previously (4), although it remains possible that this sustained component is due to the activation of another  $\text{Ca}^{2+}$ -activated current.

#### Regional Differences in the Response to Acidosis

Recent studies have shown marked heterogeneity in the electrophysiological properties of the epicardium and endocardium of the ventricle in several species,



including the rat (29). Differences in action potential configuration in the subepicardial and subendocardial regions of the ventricle have been attributed to the prominence of  $I_{to}$  in the subepicardium and its relative absence in the subendocardium (21; also see the introduction). Consistent with this view, we found that  $I_{to}$  was more marked in subepicardial cells than in subendocardial cells (Fig. 1) and that acidosis increased  $I_{to}$  in subepicardial, but not in subendocardial, cells when the holding potential was  $-40$  mV; the greater effects of acidosis in the subepicardium may be due to the greater density of  $I_{to}$  in subepicardial cells.

Antzelevitch et al. (3) reported that simulated ischemia (6 mM  $K^+$ , 95%  $N_2$ -5%  $CO_2$ ; pH 6.8) caused a marked depression of the subepicardial action potential, which could be reversed by 4-AP, but had little effect on the subendocardial action potential. This suggests that an increase in  $I_{to}$  may play a role in the observed changes in action potential configuration and that the regional differences in the response to such "ischemia" may reflect regional differences in the density of  $I_{to}$ . Our data are consistent with this hypothesis and suggest that the acid component of the simulated ischemia may be important in the response to this intervention. Thus the increase in  $I_{to}$  that we observed during acidosis in subepicardial cells may explain the depression of the subepicardial action potential observed during simulated ischemia, and the lack of effect of acidosis on  $I_{to}$  in subendocardial cells may explain why the subendocardial action potential was little affected by simulated ischemia.

In contrast to the present data, Xu and Rozanski (31) reported that lowering  $pH_i$  decreases  $I_{to}$  in rat ventricular myocytes. However, acidosis and control data were obtained in different groups of cells. Because there is a marked heterogeneity in the amplitude of  $I_{to}$  in different regions of the heart and the authors did not separate epicardial and endocardial cells, they could not rule out the possibility that the observed difference could be due to the different cell types being predominant in each population (acid and control). In addition, these authors only observed a decrease when  $pH_i$  was lowered by dialysis with an acid (pH 6.0) pipette solution. When the pH of the bathing solution was lowered, as in the present study, acidosis did not significantly alter  $I_{to}$  when recorded from a holding potential of  $-80$  mV, consistent with the results of the present study.

The present data show that acidosis increases  $I_{to}$  when the diastolic membrane potential is lower than the normal ventricular diastolic potential. This suggests that acidosis is most likely to increase  $I_{to}$  in regions of the heart where the diastolic potential is relatively positive, e.g., in the sinoatrial and atrioventricular nodes, or in pathological conditions, such as myocardial ischemia, in which an accumulation of extracellular potassium results in depolarization of the cell membrane. This suggests that either global acidosis (e.g., resulting from systemic acidosis) or regional acidosis (e.g., that found in the ischemic region during myocardial ischemia) may increase  $I_{to}$  in regions of the

heart that, either physiologically or pathophysiologically, have a depolarized membrane and are exposed to the acidosis.

Thus the data from the present study suggest that acidosis will not increase  $I_{to}$  uniformly throughout the heart for a number of reasons: 1) because  $I_{to}$  is not uniformly distributed throughout the heart; 2) because diastolic membrane potential, which influences the response of  $I_{to}$  to acidosis, is not uniform throughout the heart; and 3) some types of acidosis found in vivo, e.g., that associated with myocardial ischemia, are localized within the heart. If acidosis does not increase  $I_{to}$  uniformly throughout the heart, this will alter action potential configuration differently in different regions of the heart, thus altering action potential dispersion, which can alter the normal spread of the action potential and may lead to arrhythmias. Thus it appears that the acidosis-induced increase in  $I_{to}$  observed in the present study may have marked effects on the normal electrical activity of the intact heart.

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