On the Role of Sodium Ions in the Regulation of the Inward-Rectifying Potassium Conductance in Cat Ventricular Myocytes

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ABSTRACT The conductance of the inward-rectifying K⁺ current (I₉) in isolated cat ventricular myocytes is decreased by reducing the extracellular Na⁺ concentration. Using a whole-cell patch-clamp technique, possible mechanisms underlying this Na⁺ dependence were investigated. These included (a) block of inward K⁺ current by the Na⁺ substitute, (b) changes in membrane surface charge associated with removal of extracellular Na⁺, (c) increases of intracellular Ca²⁺ due to suppression of Na-Ca exchange, (d) reduction of a Na⁺-dependent K⁺ conductance due to a subsequent decrease of intracellular Na⁺, (e) reduction of I₉ conductance (g₉) associated with reduction of intracellular pH due to suppression of Na-proton exchange. The findings support the hypothesis that the effect of removing Na⁺ is mediated through a decrease in intracellular pH. These include observations that: (a) reducing internal pH by reducing external pH caused a decrease in g₉, and the conductance changes caused by reducing extracellular pH and removing extracellular Na⁺ were not additive; (b) the effect of reducing pHₒ was attenuated by dialyzing with a low pH internal solution; (c) g₉ was reduced by exposure to the Na-proton exchange inhibitor dimethylamiloride, and this effect was absent in the absence of Na⁺. These findings imply that physiological or pathological processes such as ischemia and metabolic or respiratory acidosis which can produce intracellular acidosis should be expected to affect K⁺ permeation through the I₉ channel.

INTRODUCTION

Of the several electrophysiological characteristics of the inward-rectifying potassium current (I₉) that have recently been described for cat ventricular myocytes, an apparent dependence of the conductance on extracellular Na⁺ (Nax) concentration is one of the more intriguing. Nax appears to exert an effect that enhances or facilitates the conductance of inward-rectifying K channels; total removal of Nax can reduce inward directed K⁺ conductance to ~60% of normal but does not eliminate
inward rectification (Harvey and Ten Eick, 1988). A similar phenomenon has been observed in guinea pig ventricular myocytes (Biermans et al., 1987). Despite the fact that this effect of Na⁺ may have important implications for our understanding of the partially depolarized resting potentials reported to occur in human atrial tissues (Ten Eick and Singer, 1979) and the propensity of diseased heart to rhythm disturbances, the underlying mechanism is yet to be elucidated.

That the conductance of IK₁ is sensitive to changes in Na⁺ concentration should not be surprising because the conductance of inward-rectifying K⁺ current found in noncardiac preparations also exhibits Na⁺ dependence. For example, the conductance of inward K⁺ current in tunicate egg cells was reduced when Na⁺ was removed (Ohmori, 1978). Although the mechanism(s) is (are) not understood, because of the increase in apparent channel activity seen using fluctuation analysis, Ohmori (1980) suggested that channel activation (as well as inactivation) exhibits Na⁺ dependence. Fukushima (1982), however, has shown that Na⁺ facilitates single-channel conductance. This has also been described in rat ventricular myocytes (Payet et al., 1985). While alterations of channel properties such as these might explain the changes observed in whole-cell currents, there are several alternative mechanisms that might also underlie the ability of extracellular Na⁺ to modulate or regulate IK₁ conductance.

Na⁺ is importantly associated with many processes underlying excitability in most tissues comprising the mammalian heart. In ventricular cells, stimulation causes the sarcolemmal permeability to Na⁺ to increase enabling Na⁺ to carry current into the cell and produce the upstroke of the action potential (Draper and Weidmann, 1951). The large gradient between extracellular and intracellular Na⁺ concentrations is also important for several ion exchange processes. The potential energy in the transmembrane Na⁺ concentration gradient is used by the Na-Ca exchanger to extrude intracellular Ca²⁺ and contribute to the homeostatic regulation of intracellular Ca²⁺ activity, thereby modulating the contraction process (Horackova and Vassort, 1979). In addition, the Na⁺ gradient is involved with an exchange process that plays an important part in the regulation of intracellular pH (see Roos and Boron, 1981). In cardiac preparations, Na-proton exchange appears to have a particularly important role in the recovery from intracellular acidosis (Deitmer and Ellis, 1980; Wallert and Frohlich, 1987). Furthermore, under normal conditions Na-H exchange may be the principal influx route for the intracellular accumulation of Na⁺ (Lazdunski et al., 1985). Finally, intracellular Na⁺ has been associated with modulation of the conductance properties of at least one type of inward-rectifying K⁺ channel in heart cells (Kameyama et al., 1984). Therefore, it is not unreasonable to suggest that reducing the Na⁺ concentration could alter IK₁ conductance by any of several mechanisms.

The hypothesis best supported by the experiments reported here is that removing Na⁺ reduces the inward-rectifying K⁺ conductance because of an acidification of the intracellular pH, arising from the inhibition of Na-proton exchange. The inward-rectifying K⁺ currents in starfish egg cells and frog skeletal muscle have been shown to be particularly sensitive to changes in internal pH. To wit, increasing intracellular proton concentration decreases the size of the inward K⁺ current (Hagiwara et al., 1978; Moody and Hagiwara, 1982; Blatz, 1984). In addition, Sato et al. (1985)
reported that the membrane conductance of guinea pig ventricular cells decreased during intracellular perfusion with acidic solution. Therefore, an intracellular acidification of the cat ventricular myocytes should be expected to decrease the conductance of $I_{K1}$. A preliminary report of this work has appeared as an abstract (Ten Eick et al., 1988).

METHODS

Preparation of Myocytes

Cat ventricular myocytes were obtained using a modification of the enzymatic (0.15% class II collagenase, Cooper Biomedical, Malvern, PA) isolation method of Silver et al. (1983), which we have described in detail elsewhere (Harvey and Ten Eick, 1988). Only quiescent rod-shaped cells that exhibited clear striations and unblemished membranes were used. Findings that the action potentials recorded in 5.4 mM K+ Tyrode's solution with 40-70 MΩ microelectrodes appeared normally configured and had resting potentials that ranged from −68 to −76 mV (not shown) suggest that the electrophysiological integrity of these cells was intact.

Voltage-Clamp Technique

Whole-cell membrane current was measured using the whole-cell patch-clamp technique as described by Hamill et al. (1981). Suction pipettes were manufactured from borosilicate glass capillary tubing with an inner diameter of 0.8–1.1 mm (Kimble Products, Toledo, OH) using a two-stage vertical pipette puller (Narishige, Tokyo, Japan). When the pipettes were filled with internal solution, they exhibited resistances that ranged between 0.3 and 0.9 MΩ; when placed in external solution, liquid junction potentials ranged between −7 and −10 mV. However, data were corrected for this effect by zeroing current passing through the pipette while the tip was placed in the appropriate internal solution before each experiment. Current recordings were obtained using the whole-cell voltage-clamp circuit of Yoshii (Narahashi et al., 1987). Inverted voltage-clamp pulses were applied to the external solution bathing the cell through a Ag-AgCl pellet/KCl-agar bridge; the cell interior was maintained at ground potential. Currents were filtered at a frequency of 2.8 kHz, recorded digitally, and stored for later analysis on magnetic disk using a PDP 11/73 computer (Digital Equipment Corp., Marlboro, MA).

Series resistance was measured and compensated for as described previously (Harvey and Ten Eick, 1988). Currents were used to make quantitative measurements (e.g., activation time constants) only if the peak current being evaluated was 4 nA or less. This would result in voltage drop of <4 mV with a series resistance of 0.5–1.0 MΩ. In addition, the activation rate of $I_{K1}$ is not steeply voltage dependent (Harvey and Ten Eick, 1987, 1988). Therefore, only small errors should be expected.

Data Analysis

Current amplitude was measured relative to the zero-current level. Peak current was defined as the maximum inward current elicited during a 75-ms test pulse. Total membrane capacitance ($C$) was calculated using the equation $C = Q/V$ where the total charge ($Q$) moved during a 10-mV depolarizing pulse was calculated by integrating the area under the capacitive transient. $I_{K1}$ conductance was calculated from the slope of the linear portion of the peak current-voltage relationship at potentials negative to its reversal. Time constants of currents following exponential time courses were estimated by computer, using a nonlinear, sum-of-least-squares fitting routine. Rising and falling phases were fit simultaneously when appropriate.
Solutions

Table I provides listings of the constituents of solutions used. In experiments where extracellular concentrations of Na⁺ were changed, [Na⁺]ₒ was varied between 0 and 125 mM. The other constituents were the same as in the control solution and osmolarity was balanced with tetramethylammonium (TMA) chloride. The pH of most external solutions was adjusted to the desired level using NaOH. Unless otherwise indicated, the external pH was 7.4. Using NaOH to raise the pH to this level added ~2.5 mM Na⁺ to the solutions; this additional Na⁺ is included in the reported concentrations. Some experiments were performed using solutions in which the pH was adjusted using TMA-OH. The pH of most internal solutions was adjusted to 7.2 using KOH. The temperature dependence of the H⁺-buffering properties of solutions containing HEPES and Tris was accounted for when adjusting pH. Control conditions were defined as those in which control extracellular and intracellular solutions were used (see Table I).

Solutions perfused the bath (volume = 2.0 ml) at a rate of 1–2 ml/min. Although the bath solution could be completely changed in <5 min, data were not acquired for 8 min after a solution change. Temperatures were maintained at 32 ± 1°C using a Peltier thermo-electric device.

**TABLE I**

Composition of Solutions

| Solutions                  | Na⁺ | K⁺ | CaCl₂ | MgSO₄ | NaH₂PO₄ | glucose | NaHCO₃ |
|----------------------------|-----|----|-------|-------|---------|---------|--------|
| Krebs-Henseliet buffer     | 130 | 4.8| 1.2  | 1.2  | 12.5  | 25      |         |
| Extracellular solutions    |     |    |       |       |        |         |        |
| Control                    | 140 | 5.4| 2.5  | 0.5  | 5.5    | 5.0     | 140    |
| TMA                        |     | 5.4| 2.5  | 0.5  | 5.5    | 5.0     | 140    |
| Li                         |     | 5.4| 2.5  | 0.5  | 5.5    | 5.0     | 140    |
| Tris                       |     | 5.4| 2.5  | 0.5  | 5.5    | 5.0     | 140    |
| Low Ca²⁺                   | 140 | 5.4| 0.5  | 0.5  | 5.5    | 5.0     | 140    |
| Low Ca²⁺/High Mg²⁺         | 140 | 5.4| 0.5  | 2.5  | 5.5    | 5.0     | 140    |
| Zero Na⁺/Zero Ca²⁺         | 5.4 |    | 0.5  | 5.5  | 5.0    | 140     |         |

| Intracellular solutions    |     |    |       |       |        |         |        |
| K-glut²                   | 130 | 10 | 10    | 1     | 1      | 5       | 5      |
| Low K⁺                    | 70  | 10 | 10    | 1     | 1      | 5       | 5      |
| BAPTA                     | 130 | 10 | 10    | 1     | 1      | 5       | 5      |
| Zero Na⁺                  | 130 | 10 | 1    | 1     | 1      | 5       | 5      |
| Low pH                    | 70  | 10 | 10    | 1     | 1      | 5       | 5      |

* N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid.
* Tetramethylammonium chloride.
* Tris(hydroxymethyl)aminomethane HCl.
* Glutamic acid (monopotassium salt).
* Ethyleneglycol-bis(β-aminoethyl ether)N,N,N',N'-tetraacetic acid.
* 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid.
* Glutamic acid (tetramethylammonium salt).
* 10 mM PIPES [piperazine-N,N'-bis(2-ethanesulfonic acid)].
RESULTS

Effect of Na⁺ on I_{K₁}

Inward-rectifying K⁺ current can be elicited from cat ventricular myocytes using a voltage-clamp protocol that holds the membrane potential at −40 mV and steps it to hyperpolarizing levels. Under control conditions, the inward current produced by test pulses to potentials more negative than E_K activates rapidly, reaching a peak within 10 ms. At potentials negative to −120 mV, the current, after reaching a peak, decays via a process resembling inactivation, to a steady-state that is reached before the end of the 75-ms test pulse (Harvey and Ten Eick, 1988).

When the extracellular Na⁺ concentration was reduced by equimolar replacement with TMA, the amplitude of the inward current was reduced. This is illustrated in Fig. 1. Panel A shows the current elicited by a test pulse to −100 mV from a cell that was exposed to various external Na⁺ concentrations. Panel B shows the peak current-voltage relationships obtained from the same cell. It can be seen that the reversal potential was not significantly shifted, as would be expected if an ion being conducted through I_{K₁} channels had been removed. It also appears that the effect of removing Na⁺ on the current amplitude was not voltage dependent because the peak I-V relationships remained essentially linear at potentials negative to E_K. However, I_{K₁} was not completely blocked by exposure to nominally Na⁺-free conditions.

The dependence of the slope conductance of peak I_{K₁} on the extracellular Na⁺ concentration is illustrated in Fig. 2. Values for slope conductances were derived from the linear inward portions of the I-V curves. The normalized values from five...
cells (each serving as its own control) are plotted as a function of the extracellular Na⁺ concentration. The relationship appears to be linear between 0 and 127.5 mM and clearly indicates that the Na⁺ concentration can influence the inward-rectifying K⁺ conductance in cat ventricular myocytes. The explanation for these findings is not immediately evident owing to the fact that exposure to Na⁺-free solutions is expected to have several rather profound effects on excitable tissues.

*Effect on Activation Rate*

If the rate of I\textsubscript{K1} activation were affected by Na\textsubscript{o}, such an effect could contribute to the apparent dependence of the conductance on Na\textsubscript{o}. This possibility was examined by determining the effect of Na\textsubscript{o} removal on the voltage dependence of the time constant for the activation of I\textsubscript{K1}. The activation rate was found to be essentially Na\textsubscript{o} independent. The result obtained from a representative myocyte is shown in Fig. 3, which indicates that the time course of the activation process was not altered by reducing Na\textsubscript{o} from 127.5 to 2.5 mM. This finding indicates that the determinants of the activation process are insensitive to whatever it is that Na⁺ does to alter the conductance.


**Na Substitutes**

A simple explanation for the Na dependence of $g_{k1}$ would be that the ion serving as the osmotic replacement for Na acts as a K channel blocker, as suggested by Biermans et al. (1987). This is not an unreasonable possibility because TMA is structurally analogous to tetraethylammonium, a well-documented blocker of potassium channels in nerve as well as in muscle (Stanfield, 1983). To test this possibility, five different Na replacements were used; as many as four were applied to some cells. Fig. 4 shows the results from a representative cell. I-V relationships were obtained under control conditions and during subsequent exposure to solutions containing 145 mM TMA, Li, or Tris as the Na replacement ion. In between each trial, the cell was exposed to control conditions and the current amplitude was returned to control levels. Similar results were seen when choline and N-methyl-D-glucamine were used as well. The reduction of $I_{k1}$ conductance was observed in every case regardless of the Na substitute used. These data suggest that the reduction of $I_{k1}$ occurred because $g_{k1}$ is sensitive to the removal of Na+ rather than to the addition of the moiety serving as the Na+ replacement.

![Figure 4](http://rupress.org/jgp/article-pdf/94/2/329/1242213/329.pdf)

**Figure 4.** $I_{k1}$ sensitivity to various Na+ substitutes. Extracellular Na+ was replaced with the ionic substitutes Tris, Li+, and TMA. Recovery of the inward current upon return of the control extracellular solution was observed between each trial. Substitutions for Na+ were equimolar; pH was adjusted with NaOH. Control intracellular solution.

**Surface Charge Effects**

Another possible explanation for the Na dependence of $g_{k1}$ is that membrane surface charge may be altered by removing extracellular Na+. Under normal conditions extracellular Na+ may be “binding” to charged membrane components, neutralizing negative external surface charge, and thereby altering the potential difference sensed by the channels (Hille, 1984). Surface charge effects are most often associated with changes in extracellular divalent cation concentrations. However, shifts in channel gating attributed to changes in surface potential have been seen in nerve when the total monovalent ion concentration is altered (Hille et al., 1975). To examine the role of changes in surface charge on the Na+ dependence of $g_{k1}$, the extracellular Ca$^{2+}$ concentration was altered to produce a predictable change in the surface charge (Hille et al., 1975).

A fivefold reduction of extracellular Ca$^{2+}$ did affect the inward-rectifying K+ current but in a manner opposite to that seen when Na+ was removed (Fig. 5). To verify that this Ca$^{2+}$-dependent change was indeed a surface charge effect rather than
some other effect mediated by the change in the Ca\(^{2+}\) concentration, Ca\(^{2+}\) was replaced with Mg\(^{2+}\). Mg\(^{2+}\) is expected to affect surface charge in a manner similar to Ca\(^{2+}\) (Hille et al., 1975). The peak I-V relationship was insensitive to its solution being changed from one containing 2.5 mM Ca\(^{2+}\) and 0.5 mM Mg\(^{2+}\) to one containing 0.5 Ca\(^{2+}\) and 2.5 mM Mg\(^{2+}\). This finding is in contrast to the result obtained when the total concentration of divalent cation was reduced from 3 to 1 mM. These findings suggest that reduction of I\(_{K1}\) conductance seen upon removal of Na\(^{+}\) cannot be an effect produced by a change in membrane surface charge associated with Na\(^{+}\) removal.

The conclusion that the change of I\(_{K1}\) conductance upon removal of Na\(^{+}\) is not due to surface charge could also be supported by the fact that Na\(^{+}\) was replaced by positively charged substitutes, in which case, assuming the replacement ions are equally effective at screening surface charges, no difference would be expected. In guinea pig ventricular myocytes (Biermans et al., 1987) using an uncharged substitute (sucrose) did not result in a decrease of I\(_{K1}\) conductance. In this case, the change in surface charge expected because of removal of Na\(^{+}\) conceivably could offset at least some of the decrease in conductance associated with a Na\(^{+}\)-dependent mechanism.

**Na-Ca Exchange**

Because extracellular Na\(^{+}\) has an important role in the regulation of intracellular Ca\(^{2+}\) (Ca\(_i\)) in cardiac myocytes, removing Na\(^{+}\) could cause an increase in Ca\(_i\) activity which in turn could underlie the Na\(^{+}\) dependence of g\(_{K1}\). Although increases in Ca\(_i\) are most often associated with activation of K\(^+\) channels (Callewaert et al., 1986), which would be expected to increase conductance, recently it has been shown that intracellular Ca\(^{2+}\) as well as other divalent cations can block K\(^+\) channels (Findlay, 1987). In the experiments we have thus far reported, cells were dialyzed with an internal solution containing 5 mM EGTA. In the absence of Na-Ca exchange the buffering capability of this Ca\(^{2+}\) chelator may have been challenged, resulting in increased Ca\(_i\) activity. Therefore, if the Na\(^{+}\) dependence of g\(_{K1}\) were mediated by an increase in Ca\(_i\) activity, increasing the Ca\(^{2+}\) buffering efficiency should alter the Na\(^{+}\) dependence of g\(_{K1}\). This idea was tested by replacing the EGTA in the internal solution with BAPTA. The affinity of this chelating agent for Ca\(^{2+}\) is greater than that of
EGTA (Tsien, 1980). Despite the superior properties of BAPTA, when Na_o was reduced from 125 to 12.5 mM, I_{K_I} conductance still decreased (Fig. 6 A). This suggests that an increase in Ca^2+ activity in response to decreasing Na_o does not have an important role in the Na_o dependence of I_{K_I}.

A possible role for an increase in Ca^2+ activity was further tested by exposing cells to Na^+ -free external solution, followed by exposure to a Na^+- and Ca^2+-free external solution. This maneuver is expected to eliminate the most likely source of Ca, which cells cannot remove in the absence of a functioning Na-Ca exchange (Ellis and MacLeod, 1985; Vaughan-Jones et al., 1983). Fig. 6 B shows that exposure to Ca^2+-free external solution caused a minor increase in the magnitude of the inward current, but the bulk of the effect induced by removal of Na_o was not reversed. These results are consistent with the results of the BAPTA experiments and support the idea that the Na_o dependence of the conductance does not significantly involve either intra- or extracellular Ca^2+.

Dependence of I_{K_I} Conductance on Intracellular Na

In guinea pig ventricular myocytes three different K^+ channels that exhibit inward-rectifying properties have been identified and characterized. These are the inward-rectifying K channel (Sakmann and Trube, 1984a, b; Kurachi, 1985), the ATP-sensitive K channel (Noma, 1983), and the Na^+-sensitive K channel (Kameyama et al., 1985).
1984). A K channel that exhibits a dependence on intracellular Na\(^+\) should not be unexpected because the inward-rectifying K\(^+\) current in starfish egg cells exhibits a well-defined dependence on the intracellular Na\(^+\) concentration (Hagiwara and Yoshii, 1979). Knowing this, it might then seem reasonable to suggest that the Na\(_o\) dependence of IK\(_l\) in cat ventricular myocytes arises from the decrease in the internal Na\(^+\) activity expected in response to Na\(_o\) removal. Because the cells were dialyzed with a fixed Na\(^+\) concentration, this does not seem very likely (Mogul et al., 1987). However, the level of Na\(^+\) at the internal surface of the sarcolemma may not be well controlled, and reducing extracellular Na\(^+\) may eliminate an inward leak (Ellis, 1977; Ellis and MacLeod, 1985), thus reducing the effective Na\(^+\) concentration at some internal site putatively controlling at least some fraction of g\(_{Kl}\).

In an attempt to assess the plausibility of such a mechanism, cells were exposed to monensin, an ionophore for monovalent cations with a very high specificity for Na\(^+\) (Pressman, 1976). Fig. 7 shows the results of a typical experiment. Cells were first exposed to a reduced external Na\(_o\) concentration (62.5 mM) to decrease the Na\(_o\) concentration at the putative internal Na\(^+\) site involved with the Na\(_o\)-dependent conductance mechanism to a level at which the K\(^+\) conductance would be less than maximal. After the current reached a steady-state level, the cells were then exposed to a 62.5 mM Na\(^+\) solution containing 10 \(\mu\)M monensin. Although the Na\(_o\) concentration was reduced, the gradient between internal and external Na\(^+\) activities would still favor transmembrane Na\(^+\) influx. If Na\(^+\) activity at or near the membrane importantly influences K\(^+\) conductance, the reduction in conductance seen in response to reducing Na\(_o\) to 62.5 mM should have been reversed when monensin was introduced. It was not reversed.

These results are also consistent with the findings of several experiments performed using a Na\(^+\)-free internal solution. Although this was not expected to eliminate an inward leak of Na\(^+\), the increased steepness of the Na\(^+\) concentration gradient within the cell (arising from the Na\(^+\)-free pipette solution) is expected to increase the efflux of Na\(^+\) from the internal sarcolemmal surface and decrease the Na\(^+\) activity at and near this surface (relative to what the activity would be when the cells are dialyzed with an internal solution containing 10 mM Na\(^+\)). When the peak slope conductances for six cells dialyzed with Na\(^+\)-free internal solution were calculated and plotted as a function of total cell capacitance, the conductances fell within the range defined by cells dialyzed with 10 mM Na\(^+\). This suggests that the Na\(_o\)
dependence of the inward-rectifying K⁺ conductance does not importantly involve changes in intracellular Na⁺ activity from 10 or more mM to something expected to be <10 mM.

**Na-H Exchange**

Intracellular pH has been identified as an important modulator of the inward-rectifying K⁺ current in starfish egg cells and frog skeletal muscle (Blatz, 1984; Hagiwara et al., 1978; Moody and Hagiwara, 1982). If the inward-rectifying K⁺ conductance in cat ventricular myocytes were similarly sensitive to intracellular pH, and if removal of extracellular Na⁺ were to decrease the rate of the Na-proton exchange and thereby provoke an intracellular acidification, ⁹ₖ should be expected to decrease when Naₐ is reduced.

**Removal of extracellular Na⁺** has been associated with intracellular acidification in sheep Purkinje fibers (Ellis and MacLeod, 1985) and cultured chick ventricular cells (Lazdunski et al., 1985; Kim et al., 1987). This is consistent with the results in rat ventricular cells and cultured chick heart cells which show an intracellular acidification upon exposure to Na-H exchange inhibitors (Lazdunski et al., 1985; Wallert and Frohlich, 1987). Therefore, the effect of the Na-H exchange inhibitor dimethylamiloride (DMA) (Vigne et al., 1982) was examined in the cat ventricular myocytes. Exposure to 1 and 10 μM DMA resulted in an apparent concentration-dependent decrease in peak inward K⁺ current at voltages negative to E_K (Fig. 8). This effect could be reversed upon washout of the drug.
This finding is consistent with the hypothesis that the Na₉ dependence of gK₁ is associated with inhibition or elimination of Na-H exchange, and possibly caused by intracellular acidification. However, DMA is an analogue of amiloride, which is known to have a variety of effects including direct drug-channel interactions (Sari-ban-Sohraby and Benos, 1986). To determine if the DMA-induced reduction of I_K₁ is caused by DMA acting as a channel blocker, the effect of DMA on I_K₁ was determined in the absence of extracellular Na⁺. Exposure of myocytes to Na⁺-free external solution prior to introduction of DMA would have eliminated Na-H exchange so that the subsequent exposure to DMA should not result in any further reduction of intracellular pH. Any additional decrease in peak I_K₁ in response to DMA exposure would be ascribed to block of K⁺ channels by DMA directly. Adding 1 μM DMA in the absence of Na⁺ did not result in any further reduction of peak I_K₁ (Fig. 9 A). However, 10 μM DMA did cause an additional decrease of peak K⁺ current (Fig. 9 B). These data support the notion that 1 μM DMA decreased gK₁ by a mechanism involving inhibition of Na-H exchange, but at the higher concentration DMA also can exhibit channel-blocking properties that are independent of its effect on Na-proton exchange. The difference between the effects of 1 and 10 μM DMA applied under control conditions (i.e., Na⁺ present; see Fig. 8) should not be totally ascribed to K⁺ channel block. It is likely that some additional inhibition of the Na-proton exchange was induced by the increase in DMA concentration. In isolated chick and rat cardiac cells, Frelin et al. (1984) reported that DMA inhibited Na/H exchange activity at concentrations greater than 5 × 10⁻⁸ M, with maximal inhibition with
10^{-5} M (K_{0.5} = 3 \times 10^{-7} M). Their results were obtained in the presence of 3 mM extracellular Na^+. Because the effectiveness of amiloride derivatives is reduced at higher Na^+ concentrations (Lazdunski et al., 1985), a slightly higher K_{0.5} would be expected under the conditions used in our experiments. Therefore, a graded response to DMA in the range of concentrations we have used would be expected and would explain the modest response at 1 \mu M.

**Low Extracellular pH**

The results of the previous experiments are consistent with the idea that the attenuation of I_{K1} by DMA was the result of an intracellular acidification. To further test the idea that a reduction of internal pH (pH_i) could be responsible for reducing I_{K1}, conductance, pH_i was reduced by exposing myocytes to a low external pH (pH_o). When pH_o was decreased from 7.4 to 5.4 (Fig. 10 A), I_{K1} was reduced to an extent which was similar to that seen when most Na_o was removed. In addition, the conductance changes associated with removing Na_o and reducing pH_o were not additive. When extracellular Na^+ was removed while maintaining a low extracellular pH, I_{K1} conductance was not significantly altered (Fig. 10 B). This suggests that both manipulations affect I_{K1} conductance through the same mechanism.

**Reduced Intracellular pH**

The experiments described immediately above were done using an intracellular solution with a pH of 7.2. It was assumed that the effect of reducing pH_o was
mediated through a change in pH, even though the cells had been dialyzed with an internal solution buffered with 5 mM HEPES. To verify that a change in intracellular H+ concentration could be responsible for the effect seen when pHo was reduced, the results of a series of experiments performed using an intracellular solution buffered with 10 mM PIPES at pH 6.8 were compared with those obtained with 5 mM HEPES at pH 7.2 (pK of HEPES = 7.4, pK of PIPES = 6.8). If the response to extracellular acidification is blunted by the more acidic internal solution, the results of this series would support the assumptions that reducing pHo reduces pH and that peak Iki can be reduced by decreasing pH.

The dependence of Iki on extracellular pH was characterized from the change in conductance observed when pHo was varied between 6.0 and 8.0. The results (Fig. 11) show that the conductance of Iki was particularly sensitive to changes in pHo between 7.4 and 7.0 when pH was 7.2. Iki conductance decreased by ~50% when pHo was reduced to 7.0; further reduction of pHo had relatively little effect. This steep pHo dependence was not seen when the experimental protocol was repeated with cells dialyzed with the more acidic PIPES-buffered intracellular solution. With the PIPES-buffered internal solution, even at pHo as low as 6.0 conductance was reduced only ~25% relative to its level when pHo was 7.4. Because dialyzing the cells with the more acidic internal solution was expected to reduce initial Iki conductance, the hypothesis predicts that subsequent reduction of pHo should have a less profound effect than that seen when pH was 7.2. Whatever the true nature of the pH dependence of Iki, these results are consistent with the idea that changes in pHo exert effects on Iki through changes in pH.

**DISCUSSION**

The decrease in Iki conductance associated with removal of extracellular Na+ is opposite to what would be predicted if Na+ were an Iki channel blocker. The simple explanation would be that Na+ regulates the conductance of the inward-rectifying K+ current. However, the consequences of removing Na+ involve several processes known to be critical for normal cellular electrophysiological function.
Changes in Intracellular pH

We examined the hypothesis that the Na\textsubscript{0} dependence of I\textsubscript{K1} conductance in cat ventricular myocytes was due to a change in intracellular pH caused by the attenuation of the Na-proton exchange associated with Na\textsubscript{0} removal. This idea assumes that myocytes are sufficiently metabolically active, so that under conditions that inhibit the normal mechanisms for maintaining intracellular pH (pHi) there would be a source of protons sufficient to effect a decrease in pHi. Under normal conditions protons are expected to be continuously generated as a by-product of the intense metabolic activity of cardiac cells (Gevers, 1977). If protons were passively distributed across the sarcolemma, with an external pH of 7.4 and a resting potential of -85 mV, an intracellular pH of ~6.0 would be expected. However, this is well below the measured intracellular pH of cardiac cells, which, depending on the preparation and the method of measurement, varies between 6.9 and 7.3 (Roos and Boron, 1981). Although cardiac cells have an intrinsic ability to buffer a large quantity of cytosolic protons, intracellular pH is maintained predominantly by two H\textsuperscript{+} extrusion mechanisms. First, using the energy of the transsarcolemmal Na\textsuperscript{+} gradient, the Na-proton exchange couples Na\textsuperscript{+} influx to proton efflux to extrude protons from cells and maintain pHi homeostatic. (Deitmer and Ellis, 1980; Ellis and MacLeod, 1985; Lazdunski et al., 1985; Wallert and Frohlich, 1987). The second mechanism is also an exchange mechanism, but in this case extracellular Na\textsuperscript{+} is exchanged for intracellular H\textsuperscript{+} by being coupled to an exchange of extracellular HCO\textsubscript{3}\textsuperscript{-} for intracellular Cl\textsuperscript{-} (Vaughan-Jones, 1986).

In the experiments presented here the latter mechanism is probably of limited significance because the myocytes were bathed in bicarbonate-free, HEPES-buffered solutions. Therefore, the Na-H exchange mechanism should be expected to be the predominant means by which intracellular pH is maintained. However, because the whole-cell patch-clamp technique used large-mouthed (tip bore = ~3–5 μm) suction pipettes containing a HEPES-buffered internal solution, additional non-physiological mechanisms intended to hold intracellular pH nearly constant were introduced. Dialysis of the cell interior was expected to allow excess H\textsuperscript{+} to diffuse from cell to pipette and the HEPES-buffered internal solution to diffuse into the cell. For the hypothesis postulating involvement of the Na-H exchange to be correct, it must be assumed that internal dialysis with a 5 mM HEPES solution cannot "clamp" the intracellular H\textsuperscript{+} concentration of metabolically active myocytes without the assistance of a normally operating Na-H exchanger. Observations that inhibition of Na-H exchange with amiloride derivatives can produce intracellular acidification in other cardiac preparations (Lazdunski et al., 1985; Wallert and Frohlich, 1987) and that exposure of cat myocytes to DMA was able to reduce I\textsubscript{K1} conductance during this study supports this idea. The results of whole-cell patch-clamp studies using guinea pig ventricular cells to characterize the pH sensitivity of the calcium current (I\textsubscript{Ca}) (Sato et al., 1985; Irisawa and Sato, 1986), also provide support for the conclusion that in the absence of an operational Na-proton exchange, the pHi of patch-clamped myocardial cells may not be held truly fixed by the buffer system in the internal solution. It is not possible to estimate the extent to which the pHi may have decreased during the present experiments. However, using sheep Purkinje fibers,
Deitmer and Ellis (1980) found that internal pH decreased 0.23 units for every unit external pH was reduced. Using this value, our results characterizing the pH dependence of I_{K_1} (see Fig. 11) suggest that a change in pH of \~0.1 unit affects I_{K_1} conductance profoundly. In addition, this is in line with the magnitude of the pH change seen in guinea pig myocytes when Na_+ is removed (Wahlert and Frohlich, 1987). Therefore, the Na\(^+\) dependence of I_{K_1} conductance could be the result of a change in pH_\text{e}.

The pH_\text{i} sensitivity of inward-rectifying K\(^+\) currents is well documented (Hagiwara et al., 1978; Moody and Hagiwara, 1982; Blatz, 1984). Therefore, it is reasonable to suggest that intracellular acidification of cat ventricular myocytes can decrease the conductance for I_{K_1}. This was tested by inducing pH_\text{i} changes by altering pH_\text{e}. It was assumed that the resulting effects were due to internal pH changes as opposed to external pH changes. This assumption is supported by the fact that reduced pH_\text{i} decreased the relative sensitivity of I_{K_1} conductance to changes in pH_\text{e}. These findings in cardiac myocytes are consistent with those obtained from a variety of other tissues and classes of channels. This includes Na\(^+\) current in squid axon (Ehrenstein and Fishman, 1971; Bass and Moore, 1973), Ca\(^{2+}\) current in Paramecium (Umbach, 1982) and guinea pig ventricular cells (Irisawa and Sato, 1986), inward-rectifying K\(^+\) current in crayfish neurons (Moody, 1981) and frog skeletal muscle (Blatz, 1984), delayed rectifier K\(^+\) current in squid axon (Carbone et al., 1981), and acetylcholine-activated K\(^+\) current in cultured chick myoballs (Goldberg and Lass, 1983). This litany suggests that, in general, ion channels are more effectively suppressed by protons at the inside than at the outside of the membrane.

We cannot rule out the possibility that external Na\(^+\) may facilitate the conductance of single K\(^+\) channels as suggested by Fukushima (1982) and Payet et al. (1985). However, Biermans et al. (1987) have concluded that it appears unlikely that Na\(^+\) ions exert a facilitating effect on single-channel conductance. Our results strongly suggest that the Na\(^+\) dependence of I_{K_1} conductance at the whole-cell level is modulated through an effect on intracellular pH. This is supported by the reduced sensitivity of I_{K_1} to changes in extracellular Na\(^+\) when conductance is already reduced by low pH (Fig. 10 B).

**Changes in Intracellular Ca\(^{2+}\)**

It has been suggested that intracellular Ca\(^{2+}\) (Ca_\text{i}) may be important in controlling pH_\text{i} (Vaughan-Jones et al., 1983; Kim et al., 1987). Therefore, the effects of removing Na_\text{a}, while ultimately mediated through changes in pH_\text{i}, may have been secondarily due to an increase in Ca_\text{i}. This possibility is ruled out by the experiments that were used to cast doubt on the hypothesis that the Na\(^+\) dependence of I_{K_1} conductance was due to an attenuation of Na-Ca exchange. However, the mechanism could conceivably be important in the opposite sense. Ca_\text{i} is believed to affect pH_\text{i} by displacing H\(^+\) from common binding sites inside the cell (Hess and Weingart, 1980). Therefore, it follows that an increase in intracellular H\(^+\) concentration might displace intracellularly bound Ca\(^{2+}\) and, because of the pH sensitivity of the EGTA used to buffer intracellular Ca\(^{2+}\), confound the interpretation of the present experiments. EGTA has two titratable H\(^+\) binding sites with pK_a of 8.96 and 9.58 (Martell and Smith, 1974). Therefore, relatively small changes in pH around 7.0 will
significantly affect its ability to chelate Ca$^{2+}$. As a result, an increase in free Ca$_i$ caused by the experimentally induced changes in pH$_i$ could be causing the changes in the $I_{K1}$ conductance. However, the results of experiments using BAPTA as the intracellular Ca$^{2+}$ buffer make this possibility seem unlikely. BAPTA has pK$_a$ of 6.36 and 5.47 (Tsien, 1980), making its ability to buffer Ca$^{2+}$ less sensitive to pH change in the physiologically relevant range. An increase in Ca$_i$ also seems an unlikely explanation because increased Ca$_i$ is said to increase rather than decrease the conductance of the inward-rectifying K$^+$ current in starfish egg cells (Hagiwara et al., 1978), as well as on that of K$^+$ currents in cardiac Purkinje fibers (Isenberg, 1977), smooth muscle cells (Mitra and Morad, 1985), skeletal muscle cells (Barrett et al., 1982), and isolated heart cells (Gallewaert et al., 1986).

**Changes in Intracellular Na$^+$**

In starfish egg cells, an intracellular Na$^+$ dependence of the inward-rectifying K$^+$ current is well documented (Hagiwara and Yoshii, 1979). Therefore, it is not surprising that a Na$_i$-sensitive potassium channel with inward-rectifying properties has been found in guinea pig ventricular cells (Kameyama et al., 1984). However, the Na$_i$-sensitive potassium channel in those ventricular cells is distinctly different from the $I_{K1}$ channel because guinea pig $I_{K1}$ channels did not exhibit a dependence on Na$^+$. Furthermore, Na$_i$-sensitive potassium channels were not active at Na$_i$ concentrations below 30 mM. In our experiments using cat ventricular myocytes, except possibly when monensin was used, the intracellular Na$^+$ concentration was never >10 mM. Therefore, it seems unlikely that the Na$_i$ dependence of the inward K$^+$ current is mediated through an effect on a Na$_i$-sensitive potassium channel, a conclusion supported by the results of experiments during which cells were exposed to monensin.

**Significance.** Na-H exchange plays an important role in signal transduction via changes in pH$_i$ in such processes as oocyte fertilization, serum activation of cell growth and proliferation, activation of lymphocytes, neutrophils, and platelets, and insulin-induced glycolysis (see Mahnensmith and Aronson, 1985). Although these processes involve the activation of an otherwise “dormant” Na-proton exchange, they lend credibility to the idea that changes in pH$_i$ occurring in response to an attenuation of the Na-H exchange rate could have profound effects in cardiac tissue. Although changes in Na$_i$ concentration within the physiologic or even a pathologic range would probably have little effect on $I_{K1}$ conductance through this mechanism, this study indicates that cardiac inward-rectifying K$^+$ current, at least in cat ventricular myocytes, is extremely sensitive to pH change. Therefore, it is conceivable that electrophysiologic abnormalities associated with those pathologic conditions that result in an intracellular acidification could be due to changes in $I_{K1}$. For example, the depolarized resting membrane potential found in diseased human cardiac tissue (Ten Eick and Singer, 1979) may be explained by a decrease in the inward-rectifying K$^+$ conductance caused by either intracellular acidification or an altered sensitivity to intracellular protons. Similarly, decreases of $I_{K1}$ conductance induced by exposure to amphiphiles such as lysophosphatidylcholine (Clarkson and Ten Eick, 1983) could also involve intracellular acidification or altered sensitivity to protons (Snyder et al., 1981).
This work was supported by United States Public Health Service, National Heart, Lung, and Blood Institute, Cardiovascular Diseases Branch grant HL-27026, and Chicago Heart Association grant GCJ 34/32 to R. E. Ten Eick and a Northwestern University predoctoral training fellowship to R. D. Harvey.

Original version received 23 May 1988 and accepted version received 27 February 1989.

REFERENCES

Bass, L., and W. J. Moore. 1973. The role of protons in nerve conduction. Progress in Biophysics and Molecular Biology. 27:141–171.

Biermans, G., J. Vereecke, and E. Carmeliet. 1987. The mechanism of the inactivation of the inward-rectifying K current during hyperpolarizing steps in guinea pig ventricular myocytes. Pflügers Archiv. 410:604–619.

Blatz, A. L. 1984. Asymmetric proton block of inward-rectifier K channels in skeletal muscle. Pflügers Archiv. 401:402–407.

Callewaert, G., J. Vereecke, and E. Carmeliet. 1986. Existence of a calcium-dependent potassium channel in the membrane of cow cardiac Purkinje cells. Pflügers Archiv. 406:424–426.

Carbone, E., P. L. Testa, and E. Wanke. 1981. Intracellular pH and ionic channels in the Loligo vulgaris giant axon. Biophysical Journal. 35:395–413.

Clarkson, C. W., and R. E. Ten Eick. 1983. On the mechanism of lysophosphatidylcholine–induced depolarization of cat ventricular myocardium. Circulation Research. 52:543–556.

Deitmer, J. W., and D. Ellis. 1980. Interaction between the regulation of the intracellular pH and sodium activity of sheep cardiac Purkinje fibres. Journal of Physiology. 304:471–488.

Draper, M. H., and S. Weidmann. 1951. Cardiac resting and action potentials recorded with an intracellular electrode. Journal of Physiology. 115:74–94.

Ehrenstein, G., and H. M. Fishman. 1971. Evidence against hydrogen-calcium competition model for electrically excitable membranes. Nature. 233:16–17.

Ellis, D. 1977. The effects of external cations and ouabain on intracellular sodium activity of sheep heart Purkinje fibres. Journal of Physiology. 273:211–240.

Ellis, D., and K. T. MacLeod. 1985. Sodium-dependent control of intracellular pH in Purkinje fibres of sheep heart. Journal of Physiology. 359:81–105.

Findlay, I. 1987. ATP-sensitive K+ channels in rat ventricular myocytes are blocked and inactivated by internal divalent cations. Pflügers Archiv. 410:313–320.

Frelin, C., P. Vigne, and M. Lazdunski. 1984. The role of the Na'/H' exchange system in cardiac cells in relation to the control of the internal Na' concentration. Journal of Biological Chemistry. 259:8880–8885.

Fukushima, Y. 1982. Blocking kinetics of the anomalous potassium rectifier of tunicate egg studied by single channel recording. Journal of Physiology. 331:311–331.

Gevers, W. 1977. Generation of protons by metabolic processes in heart cells. Journal of Molecular and Cellular Cardiology. 9:867–874.

Goldberg, G., and Y. Lass. 1983. Evidence for acetylcholine receptor blockade by intracellular hydrogen ions in cultured chick myoballs. Journal of Physiology. 343:429–437.

Hagiwara, S., S. Miyazaki, W. Moody, and J. Patlak. 1978. Blocking effects of barium and hydrogen ions on the potassium current during anomalous rectification in the starfish egg. Journal of Physiology. 279:167–185.

Hagiwara, S., and M. Yoshii. 1979. Effects of internal potassium and sodium on the anomalous rectification of the starfish egg as examined by internal perfusion. Journal of Physiology. 292:251–265.
Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflügers Arch. 391:85–100.

Harvey, R., and R. Ten Eick. 1987. K and Na sensitivity of I(Inward-rectifying K Conductance

Harvey, R. D., and R. E. Ten Eick. 1988. Characterization of an inward rectifying potassium current in cat ventricular myocytes. Journal of General Physiology. 91:593–615.

Hess, P., and R. Weingart. 1980. Intracellular free calcium modified by pH in sheep Purkinje fibres. Journal of Physiology. 507:61P. (Abstr.)

Hille, B., A. M. Woodhull, and B. I. Shapiro. 1975. Negative surface charge near sodium channels of nerve: divalent ions, monovalent ions, and pH. Philosophical Transactions of the Royal Society, Biological Sciences. 270:301–318.

Horackova, M., and G. Vassort. 1979. Sodium-calcium exchange in regulation of cardiac contractility: evidence for an electrogenic, voltage dependent mechanism. Journal of General Physiology. 70:403–424.

Irisawa, H., and R. Sato. 1986. Intracellular Na + activates a K + channel in mammalian cardiac cells. Nature. 309:354–356.

Isenberg, G. 1977. Cardiac Purkinje fibres (Ca2+) controls the potassium permeability via the conductance components gK1 and gK2. Pflügers Archiv. 371:77–85.

Kameyama, M., M. Kakai, R. Sato, T. Shibasaki, H. Matsuda, and H. Irisawa. 1984. Intracellular Na + activates a K + channel in mammalian cardiac cells. Nature. 309:354–356.

Kim, D., E. J. Cracog, Jr., and T. W. Smith. 1987. Relations among sodium pump inhibition, Na-Ca and Na-H exchange activities, and Ca-H interaction in cultured chick heart cells. Circulation Research. 60:185–193.

Kurachi, Y. 1985. Voltage-dependent activation of the inward-rectifier potassium channel in the ventricular cell membrane of guinea-pig heart. Journal of Physiology. 366:365–385.

Lazdunski, M., C. Frelin, and P. Vigne. 1985. The sodium/hydrogen exchange system in cardiac cells: its biochemical and pharmacological properties and its role in regulating internal concentrations of sodium and internal pH. Journal of Molecular and Cellular Cardiology. 17:1029–1042.

Mahmenschmidt, R. L., and P. S. Aronson. 1985. The plasma membrane sodium-hydrogen exchanger and its role in physiological and pathophysiological processes. Circulation Research. 56:773–788.

Martell, A. E., and R. M. Smith. 1974. Critical Stability Constants. Vol. I. Plenum Publishing Co., New York.

Mitra, R., and M. Morad. 1985. Ca2+ and Ca2+-activated K+ currents in mammalian gastric smooth muscle cells. Science. 229:269–272.

Mogul, D. J., D. H. Singer, and R. E. Ten Eick. 1987. Intracellular diffusion of Na from patch electrodes in cardiac myocytes: implications for Na activity at internal Na pump sites. Biophysical Journal. 51:261a. (Abstr.)

Moody, W. J. 1981. The ionic mechanism of intracellular pH regulation in appearance of calcium action potentials in crayfish neurons. Journal of Physiology. 316:293–308.

Moody, W. J., and S. Hagiwara. 1982. Block of inward rectification by intracellular H+ in immature oocytes. Journal of General Physiology. 79:115–130.

Narahashi, T., A. Tsunoo, and M. Yoshii. 1987. Characterization of two types of calcium channels in mouse neuroblastoma cells. Journal of Physiology. 383:231–249.

Noma, A. 1983. ATP-regulated K+ channels in cardiac muscle. Nature. 305:147–148.

Ohmori, H. 1978. Inactivation kinetics and steady-state current noise in the anomalous rectifier of tunicate egg cell membranes. Journal of Physiology. 281:77–99.
Ohmori, H. 1980. Dual effects of K ions upon the inactivation of the anomalous rectifier of the egg cell membrane. Journal of Membrane Biology. 53:143–156.

Payet, M. D., E. Rousseau, and R. Sauve. 1985. Single-channel analysis of a potassium inward rectifier in myocytes of newborn rat heart. Journal of Membrane Biology. 86:79–88.

Pressman, B. C. 1976. Biological applications of ionophores. Annual Review of Biochemistry. 45:501–531.

Roos, A., and W. F. Boron. 1981. Intracellular pH. Physiological Reviews. 61:296–434.

Sakmann, B., and G. Trube. 1984a. Conductance properties of single inwardly rectifying potassium channels in ventricular cells from guinea-pig heart. Journal of Physiology. 347:641–657.

Sakmann, B., and G. Trube. 1984b. Voltage-dependent inactivation of inward-rectifying single-channel currents in the guinea-pig heart cell membrane. Journal of Physiology. 347:659–683.

Sariban-Sohraby, S., and D. J. Benos. 1986. The amiloride sensitive sodium channel. American Journal of Physiology. 250:C175–C190.

Sato, R., A. Noma, Y. Kurachi, and H. Irisawa. 1985. Effects of intracellular acidification on membrane currents in ventricular cells of the guinea pig. Circulation Research. 57:553–561.

Silver, L. H., E. L. Hemwall, T. A. Marino, and S. R. Houser. 1983. Isolation and morphology of calcium-tolerant feline ventricular myocytes. American Journal of Physiology. 245:H891–H896.

Snyder, D. W., W. A. Crafford, Jr., J. L. Glashow, D. R. Rankin, B. E. Sobel, and P. B. Corr. 1981. Lysophosphoglycerides in ischemic myocardium effluents and potentiation of their arrhythmogenic effects. American Journal of Physiology. 241:H700–H707.

Stanfield, P. R. 1983. Tetraethylammonium ions and the potassium permeability of excitable cells. Reviews of Physiology Biochemical Pharmacology. 97:1–67.

Ten Eick, R. E., E. J. Cragoe, Jr., and R. D. Harvey. 1988. Sodium and intracellular pH dependence of I_k conductance. Biophysical Journal. 53:162a. (Abstr.)

Ten Eick, R. E., and D. H. Singer. 1979. Electrophysiological properties of diseased human atrium. I. Low diastolic potential and altered cellular response to potassium. Circulation Research. 44:545–557.

Tsien, R. Y. 1980. New calcium indicators and buffers with high selectivity against magnesium and protons: design, synthesis, and properties of prototype structures. Biochemistry. 19:2396–2404.

Umbach, J. A. 1982. Changes in intracellular pH affect calcium currents in Paramecium caudatum. Proceedings of the Royal Society of London, Biological Sciences. 216:209–224.

Vaughan-Jones, R. D. 1986. An investigation of chloride-bicarbonate exchange in the sheep cardiac Purkinje fibre. Journal of Physiology. 379:377–406.

Vaughan-Jones, R. D., W. J. Lederer, and D. A. Eisner. 1983. Ca^{2+} ions can affect intracellular pH in mammalian cardiac muscle. Nature. 301:522–524.

Vigne, P., C. Frelin, and M. Lazdunski. 1982. The amiloride-sensitive Na/H exchange system in skeletal muscle cells in culture. Journal of Biological Chemistry. 257:9994–9400.

Wallart, M. A., and O. Frohlich. 1987. pH regulation in isolated ventricular myocytes. Biophysical Journal. 51:264a. (Abstr.)