Swelling-Induced Chloride-Sensitive Current in Canine Atrial Cells Revealed by Whole-Cell Patch-Clamp Method

Steve Sorota

An isoproterenol-induced chloride current has been detected in ventricular myocytes from guinea pig and rabbit but has not been found in canine ventricular cells. This investigation was undertaken to determine whether canine atrial cells possessed such a current. Steady-state currents were examined with potassium currents blocked by cesium. In whole-cell patch-clamp experiments, an isoproterenol-induced chloride current could not be detected shortly after patch rupture. However, whole-cell current in the absence of isoproterenol increased over time after patch rupture. The spontaneously activating steady-state current was outwardly rectifying with a reversal potential of approximately \(-25\) mV. The current that developed over time was sensitive to variation in extracellular chloride concentration and was partially blocked by anthracene-9-carboxylic acid. Isoproterenol could enhance the amplitude of this current once it developed. Although isosmotic pipette filling and extracellular solutions were used, cell swelling was found to be the cause of the increase in whole-cell conductance that was observed during whole-cell patch-clamp experiments. The development of the current and the associated cell swelling could be prevented with the addition of 50–75 mM mannitol to the extracellular solution. The current could be observed in perforated patch recordings with nystatin when extracellular osmolarity was low (221 mosm/kg) but not when the extracellular solution was isosmotic (293 mosm/kg). Cardiac chloride currents have the potential to depolarize the resting membrane potential and cause abnormal automaticity. Chloride currents can also decrease the refractory period through a reduction in action potential duration. The presence of a swelling-induced chloride current could be of importance for understanding cardiac electrical activity during pathological states that are associated with myocardial swelling. (Circulation Research 1992; 70:679–687)

KEY WORDS * chloride current * atrial myocytes * swelling * patch clamp * perforated patch recording

The contribution of chloride currents to cardiac electrical activity has recently been reevaluated by several groups of investigators.\(^1\)\(^-\)\(^5\) The reversal potential for chloride under physiological conditions in cardiac muscle is approximately \(-50\) mV.\(^6\)\(^-\)\(^9\) Therefore, chloride currents have the potential to contribute outward (repolarizing) current at plateau voltages and inward (depolarizing) current at normal resting potentials.\(^2\)\(^,\)\(^10\)

Present interest in the contribution of chloride current to cardiac transmembrane potentials was triggered by the observation of Egan et al\(^11\) that isoproterenol could depolarize the resting potential of isolated guinea pig ventricular myocytes. The underlying current was eventually shown to be a chloride current that was dependent on extracellular monovalent cations.\(^1\)\(^-\)\(^3\) Activation of this chloride current in guinea pig ventricular myocytes is dependent on elevation of intracellular cAMP levels and is mediated by phosphorylation.\(^1\)\(^,\)\(^12\)

The isoproterenol-stimulated chloride current does not appear to require elevation of intracellular calcium, because it is detectable when calcium currents are blocked and EGTA is present in the patch electrode.\(^2\) Single-channel recordings suggest that a low-density low-conductance (13 pS for outward currents) channel is responsible for the macroscopic current.\(^4\) In addition to the isoproterenol-enhanced chloride current, a calcium-dependent chloride current has been described in rabbit ventricular myocytes.\(^5\) This current is responsible for the 4-aminopyridine-insensitive transient outward current in these cells.\(^5\) It is not clear at this time whether there are multiple chloride channels in the heart or whether a single type of chloride channel can be activated by multiple messengers.

In contrast to guinea pig cells, when transmembrane potential has been recorded from canine ventricular myocytes with 3 M KCl-filled fine-tipped microelectrodes, an isoproterenol-induced depolarization has not been detected.\(^13\) Whole-cell patch-clamp experiments have failed to detect an isoproterenol-stimulated chloride current in canine ventricular myocytes under conditions that readily reveal this current in guinea pig ventricular myocytes.\(^13\) Much remains to be learned about the species dependence and tissue distribution of cardiac chloride currents. The purpose of this study was

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to determine whether isoproterenol could enhance a chloride current in isolated canine atrial myocytes. The results were unexpected. No chloride current was detected shortly after patch rupture. However, after the establishment of the whole-cell recording configuration, a chloride current developed over time. The spontaneously developing chloride current and the mechanism responsible for its activation are described in this article.

Materials and Methods

Canine atrial cells were isolated as previously described. Briefly, the right atrium was perfused at a pressure of 60 mm Hg with calcium-free Tyrode’s solution at 37°C supplemented with 0.1% bovine albumin (Fraction V, ICN ImmunoBiologicals, Costa Mesa, Calif.), 20 mM taurine, 5 mM mannitol, and 5 mM pyruvic acid. After 5 minutes, the same solution, with the addition of 50 μM calcium and 0.5 mg/ml collagenase (type A, Boehringer Mannheim Corp., Indianapolis, Ind.), was perfused through the tissue for 12 minutes. The tissue was then minced and placed in fresh collagenase solution supplemented with 0.05 mg/ml protease (type XXIV, Sigma Chemical Co., St. Louis, Mo.) and 0.075 mg/ml deoxyribonucleose II (D-8674, Sigma) for five 15-minute cycles. At the end of each cycle, the supernatant was removed and replaced with fresh enzyme solution. The supernatants were filtered through 200-μm nylon mesh. Cells were pelleted by centrifugation for 4 minutes at 50g, washed one time with resuspension solution (see below), and stored at room temperature in resuspension solution. Canine ventricular cells were isolated by a similar method.

For voltage-clamp recording, cells were allowed to settle onto ethanol-washed glass coverslips placed on the bottom of an 0.8-ml bath. The flow rate was approximately 4 ml/min, and the temperature in all experiments was 36±0.5°C. Cells were visualized with an inverted microscope (Nikon) at a magnification of either ×300 or ×600.

Whole-cell patch-clamp experiments were performed as previously described. After membrane rupture, the tubing connected to the electrode holder was opened to the atmosphere to ensure that pressure was not being applied to the back end of the electrode. Perforated patch (nystatin) recordings were performed as described by Horn and Marty except that a 100 mg/ml stock solution was prepared in dimethyl sulfoxide. Voltage-clamp protocols were generated, and data were acquired and analyzed using versions 5.03 and 5.5 of pCLAMP (Axon Instruments, Foster City, Calif.). Chloride currents were measured using slow hyperpolarizing ramps (−16.7 mV/sec) from +30 to −110 mV. The ramps were preceded by a 2-second step from the holding potential (−40 mV) to +30 mV. Potassium currents were blocked by intracellular and extracellular cesium. The transient current response to a 5-mV hyperpolarizing voltage step was used to estimate cell membrane capacitance and series resistance. Cell membrane capacitance was estimated by dividing the integral of this current with respect to time by the voltage step. The time constant for this current was estimated by fitting the curve to a single exponential. Series resistance was then calculated by dividing the time constant by the membrane capacitance. In these experiments, pipette voltage was nulled in the extracellular solution. Junction potentials were compensated for as previously described. Electrodes, fabricated from 1.5-mm-o.d. borosilicate capillary glass (Sutter Instrument Co., Novato, Calif.), had resistances between 2 and 4 MΩ when filled with pipette solution.

Cell width was monitored in some experiments as an indicator of cell volume. Volume changes in rat and rabbit ventricular myocytes have been found to be proportional to changes in cell width. In these experiments, total magnification of the cell was ×600. Changes in cell width were noted using a reticle in the ocular and documented by video recording.

Solutions

The modified Tyrode’s solution used for the preparation of isolated myocytes contained (mM) NaCl 144, NaHCO3 24, KC1 4, CaCl2 1.8, NaH2PO4 1.6, MgCl2 1, and dextrose 5.5. This solution was equilibrated with 95% O2–5% CO2 before use. The resuspension solution used to store isolated canine atrial cells contained (mM) NaCl 133, HEPES-NaOH 20 (pH 7.4), KCl 4.7, glucose 11, MgSO4 1.2, mannitol 5, and pyruvic acid 5, along with 20 μg/ml gentamicin and 0.1% bovine albumin.

The pipette solution used in whole-cell patch-clamp experiments contained (mM) cesium aspartate 100, CsCl 40, HEPES-CsOH 10 (pH 7.2), EGTA 5, disodium phosphocreatine 5, magnesium ATP 3, and MgCl2 1 (296 mosm/kg). In some experiments a chloride-free pipette solution was used in which cesium methanesulfonate was substituted for the aspartate and chloride salts and MgSO4 was substituted for MgCl2. The pipette solution that was used for nystatin experiments contained (mM) cesium aspartate 100, CsCl 40, HEPES-CsOH 10 (pH 7.2), CaCl2 1.8, and MgCl2 1, along with 100 μg/ml nystatin.

The extracellular solution used in most of these experiments contained (mM) NaCl 129.4, HEPES-NaOH 10 (pH 7.4), CsCl 20, CaCl2 1.8, MgCl2 1, and dextrose 5.5 (296 mosm/kg). Variations on the extracellular solution include 1) a low-chloride solution (6.6 mM) in which the methanesulfonate salts of sodium and cesium replaced the chloride salts, 2) a chloride-free solution using sodium and cesium methanesulfonate and the sulfate salts of calcium and magnesium, and 3) a low osmolarity solution with 69.7 mM NaCl. A higher concentration of calcium (2.2 mM) was used for all methanesulfonate-containing solutions. Mannitol was added to extracellular solutions as an osmotic agent where indicated. The osmolarity of the solutions used was measured by freezing-point depression (μOsmette, Precision Systems Inc., Natick, Mass.). When anthracene-9-carboxylic acid (9-AC) was used, it was dissolved directly into warm extracellular solution with prolonged stirring.

All salts used in this study were from Fisher Scientific Co., Pittsburgh, Pa. Cesium hydroxide was purchased from Aldrich Chemical Co., Milwaukee, Wis. All other chemicals were acquired from Sigma.

Results

Spontaneously Developing Steady-state Current

The ruptured patch whole-cell recording technique was used to record pseudo–steady-state currents from...
canine atrial cells. Potassium currents were blocked by using potassium-free solutions containing cesium (intracellular, 140 mM; extracellular, 20 mM). This method has previously been used to successfully record isoproterenol-stimulated chloride currents from guinea pig ventricular myocytes. Al An example of the response of canine atrial cells to 1 μM isoproterenol under these conditions is shown in Figure 1. Shortly after establishing the whole-cell configuration with a canine atrial cell, isoproterenol had no effect on steady-state current. The lack of effect on whole-cell conductance is similar to what is seen in canine ventricular cells. Thirteen minutes after patch rupture, there was still no effect of isoproterenol on steady-state whole-cell currents. Distinctly different results were obtained when this cell was exposed to isoproterenol 30 minutes after patch rupture. At this time, isoproterenol induced an outwardly rectifying current with a reversal potential near −25 mV (Figure 1).

In parallel with the time-dependent increase in the isoproterenol-induced current, there was an increase in control whole-cell conductance in the absence of isoproterenol (Figure 2). The time-dependent increase in control whole-cell conductance was also outwardly rectifying and had a reversal potential near −25 mV. The parallel time course for the increase in control and isoproterenol-stimulated currents suggests that isoproterenol modulates the current once it develops but cannot induce the current if it is not already present. Qualitatively similar results were obtained in 12 of 13 cells in this study. The time course for the development of the outwardly rectifying current was variable from cell to cell. The current developed as quickly as 5 minutes after patch rupture in some cells, but in others it took up to 25 minutes to appear. The current developed faster when the series resistance was lower.

The reversal potential for the isoproterenol-induced current and the outwardly rectifying current−voltage relation were similar to those observed for the isoproterenol-induced chloride current in guinea pig ventricular cells. Chloride concentrations in the extracellular and pipette solutions were varied to determine whether this ion was a charge carrier for the outwardly rectifying current.

**Ionic Substitution and Current Block**

When extracellular chloride was reduced to 6.6 mM after the outwardly rectifying current had developed, the zero whole-cell current voltage shifted in the positive direction, and the amplitude of the outward current (chloride influx) at positive potentials was reduced (Figure 3). Qualitatively, these changes are as expected, if the current that develops over time after patch rupture is a chloride current.

Chloride was removed from both the pipette and the extracellular solution in an attempt to block the development of the current. Contrary to expectations if the current was a pure chloride current, there was still an increase in whole-cell conductance over time (Figure 4). However, when extracellular chloride was increased to 155 mM, there was a large increase in the outward whole-cell current and a shift in the zero current voltage to more negative potentials (Figure 4). The effect of 155 mM extracellular chloride washed out on return to chloride-free extracellular solution.

Another means to characterize the ionic basis for the spontaneously developing increase in whole-cell conductance is to use selective blockers. 9-AC has been used as a blocker of cardiac chloride channels. When 1 mM 9-AC was applied after the development of an

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**Figure 1.** Time-dependent increase in isoproterenol-stimulated whole-cell current in canine atrial cells during whole-cell patch-clamp recording. Difference currents (1 μM isoproterenol−control) are plotted for various times after establishing the whole-cell patch-clamp configuration by patch rupture. At early times, isoproterenol did not induce a significant steady-state current. Thirty minutes after patch rupture, an outwardly rectifying current was induced by isoproterenol. The isoproterenol-induced current appeared only after the control whole-cell current had spontaneously increased (see Figure 2). Steady-state currents were measured with a hyperpolarizing voltage ramp at −16.7 mV/sec. Potassium currents were blocked by cesium.

**Figure 2.** Control whole-cell current (in the absence of isoproterenol [ISO]) increases over time in canine atrial cells during whole-cell patch-clamp recording. The current that develops over time is outwardly rectifying and has a reversal potential that is similar to the ISO-induced current (see Figure 1). Times listed are the minutes after patch rupture. The concentration of ISO used for the 30-minute tracing was 1 μM. Steady-state currents were measured with slow hyperpolarizing voltage ramps. Potassium currents were blocked by cesium. These tracings are from the same cell that was used for Figure 1.
outwardly rectifying whole-cell current, both inward and outward whole-cell conductances were reduced with no change in the zero current voltage (Figure 5). The effects of 9-AC on the whole-cell current–voltage relation washed out on removal of the drug. A second chloride channel blocker, 4,4’-dinitrostilbene-2,2’-disulfonic acid (DNDS) did not block this current at a concentration of 10^{-4} M (not shown). This concentration of DNDS has been reported to completely block isoproterenol-induced chloride current in guinea pig ventricular cells.1

Perforated Patch Recordings

Perforated patch recording allows the measurement of whole-cell currents without the large disruption of the cytosol that occurs during ruptured patch whole-cell recordings.17 The pore-forming antibiotic, nystatin, was used to gain electrical access to the cell interior. Nystatin forms monovalent cation-selective channels in the membrane patch under the electrode to allow electrical access to the cell interior. This technique was used to determine if chloride currents would activate when only monovalent cations, water, and, to a lesser extent, chloride were allowed to exchange between the pipette and the cytosol.17 With this technique, there is no well-defined zero time, because the access resistance gradually falls as nystatin partitions into the membrane. The time at which series resistance fell to below 20 MΩ was designated as the zero time for these experiments. An example of the results obtained with this type of recording is shown in Figure 6. No increase in whole-cell conductance was observed during a perforated patch recording lasting 35 minutes. After 35 minutes of recording, exposing the cell to isoproterenol did not increase the steady-state conductance. Similar results were obtained in four perforated patch recordings lasting 30 minutes or longer. Whole-cell currents for the beginning and the end of ruptured patch and perforated patch experiments were normalized for cell conductance and then averaged. In ruptured patch whole-cell recordings, there was a clear increase in chloride current over the time course of the experiment. In contrast, the initial and ending current–voltage relations did not differ significantly when the nystatin method was used (Figure 7).

Involvement of Cell Swelling in Chloride Current Activation

Up to this point the data had suggested that either an increase in the cytosolic concentration of an activator supplied by the pipette solution or a decrease in the concentration of an endogenous cytosolic inhibitor was the mechanism responsible for chloride current activation. Swelling seemed a less likely mechanism because there was no dramatic change in cell size or shape when viewed at ×300 magnification and because the pipette solution and the extracellular solution were isosmotic (296 mosm/kg). However, a swelling-induced current has been activated in a colonic epithelial cell line during whole-cell patch-clamp experiments when the pipette solution and bathing solution were isosmotic.21 The swelling and the resulting chloride current were prevented when the extracellular solution was made hyperosmotic by the addition of mannitol.21 This phenomenon has been attributed to the presence of low mobility osmoles within the cytoplasm.21 Because there was a precedent for a swelling-induced chloride current with isosmotic internal and external solutions21 and because cardiac cells do possess swelling-induced chloride currents,22 experiments were performed in which cell width was carefully measured at ×600 magnification during ruptured patch whole-cell recording. A detectable increase in cell width over time was seen in six of seven cells during whole-cell patch-clamp recording. An example is shown in Figure 8. The average increase in cell width was 7.8 ± 2.0 micrometers (n = 7, not shown).
width was 12±4%. Chloride current increased in all seven of these cells over the time course of the experiment. Addition of mannitol to the extracellular solution returned the cell width to its initial value (Figure 8) and simultaneously decreased whole-cell conductance toward its initial level without changing the reversal potential of the spontaneously activating current (Figure 9). Hyperosmotic extracellular solution decreased cell width and whole-cell conductance in five of five cells tested. The amount of mannitol that was required to return cell width to initial values and decrease chloride conductance was between 50 and 75 mM.

If cell swelling is the mechanism responsible for activation of canine atrial chloride current, then one must ask why a time-dependent increase in chloride conductance is not observed in canine ventricular myocytes under similar conditions. In the study that initially demonstrated a swelling-induced chloride current in canine ventricular myocytes, a pipette solution with roughly twice the osmolarity of the extracellular solution was used to induce swelling. The possibility exists that canine ventricular cells are more resistant to swelling than atrial cells. To test this hypothesis, cell width changes in response to hypotonic bathing solutions were monitored in a series of canine atrial and ventricular myocytes. Cell widths were normalized to their values in the control solution. The control solution for this study contained 69.7 mM NaCl supplemented with 110 mM mannitol. The final osmolarity of this solution was 293 mosm/kg. Cell width was measured in solutions with 74, 37, and 0 mM mannitol added (256, 221, and 185 mosm/kg, respectively). Canine atrial cells responded with a significantly (p<0.05 by Student's t test) larger percent increase in cell width than ventricular cells in each of the hypotonic solutions (Figure 10).

The electrical response of cells to hypotonic stress was studied using perforated patch recording. The control solution for this study was 69.7 mM NaCl-HEPES-buffered saline osmotically supplemented with 110 mM mannitol (293 mosm/kg). The test solution contained 37 mM mannitol (221 mosm/kg). This solution was chosen because it approximates the minimum osmotic gradient that develops across the cell membrane during whole-cell patch-clamp recording. In support of this notion, the percent increase in atrial cell width was comparable in the 37 mM mannitol solution (12±5%) to that observed during whole-cell patch-clamp recordings with isosmotic pipette and bathing solutions (12±4%). In four of five atrial cells, chloride current was induced by the hypotonic solution. In contrast, chloride current was not induced in any of five canine ventricular cells. Average whole-cell current-voltage relations, normalized for cell capacitance, are plotted in Figure 11. The hypotonic solution causes a clear increase in the chloride current in atrial cells but not in ventricular myocytes. The results of the present study suggest that canine ventricular myocytes are more resistant to osmotic stress than canine atrial cells.

**Discussion**

The initial goal of this study was to determine whether canine atrial cells possessed an isoproterenol-induced chloride current similar to that observed in guinea pig ventricular myocytes. Isoproterenol did not regulate chloride current either at short times after membrane rupture in conventional whole-cell patch-clamp experiments or at any time during perforated patch recordings. This result suggests that in healthy canine atrial cells an isoproterenol-induced chloride current is not functionally present.

While looking for an isoproterenol-induced chloride current in canine atrial cells, a current that activated spontaneously during whole-cell patch-clamp recording was noted. The current was outwardly rectifying and had a reversal potential that was consistent with it being a chloride current. Once activated, isoproterenol could
enhance the amplitude of this current. Single-channel data will be needed to determine if the channel responsible for the spontaneously activating current found in canine atrial myocytes is distinct from the β-adrenoceptor agonist–induced current found in guinea pig ventricular cells. The possibility exists that there are three distinct chloride channels in heart muscle that are responsible for 1) the β-adrenoceptor agonist–induced chloride current, 2) the 4-aminopyridine–insensitive transient outward current, and 3) the current activated in canine atrial cells during whole-cell patch-clamp recording.

Removing extracellular chloride decreased the amplitude of the outward current at positive potentials and shifted the zero current voltage for the steady-state whole-cell current–voltage relation to more positive potentials. These effects are qualitatively as expected for a chloride current. However, the magnitude of the shift in the zero current voltage suggests that the channel responsible for this current allows other ions besides chloride to pass. A positive reversal potential is expected with 40 mM intracellular chloride and 6.6 mM extracellular chloride. However, an additional complication must be considered when attempting to interpret this experiment. The intracellular chloride concentration during whole-cell patch-clamp experiments might not be solely dependent on the chloride concentration in the pipette solution. Intracellular chloride concentrations might be sensitive to a reduction in extracellular chloride. In that case, chloride reversal potential would be less positive than the theoretical value derived from the chloride concentration in the pipette solution and the extracellular solution. Measurements of intracellular chloride activity during similar whole-cell patch-clamp experiments will be necessary before this experiment can be unambiguously interpreted.

The spontaneously activating current developed with chloride-free pipette and extracellular solutions. In this case, the magnitude of the outward current at positive potentials was enhanced by elevating extracellular chloride. The zero current voltage was shifted to more negative potentials when extracellular chloride was elevated. This experiment demonstrates that elevation of intracellular chloride is not a prerequisite for activation of the current. Two aspects of this experiment merit consideration in attempting to interpret the results.

First, although the pipette solution was chloride-free initially, the solution contacted a silver–silver chloride pellet inside the pipette holder; thus, the final solution will contain some chloride. Second, sulfate and methanesulfonate were present on both sides of the membrane and may have served as charge carriers of ionic current through an anion channel under chloride-free conditions. The results of the chloride substitution experiments are consistent with the opening of an anion channel that has a higher permeability for chloride than for methanesulfonate.

The chloride channel blocker 9-AC partially inhibited the spontaneously activating current without shifting the reversal potential for this current. The lack of a change in the reversal potential in the presence of the blocker implies that the spontaneous conductance change is dominated by a single 9-AC–sensitive component. Therefore, it appears that the time-dependent increase observed in canine atrial whole-cell conductance can be attributed to a chloride channel that is not totally selective for chloride. At this time, however, the possibility that there are cation-selective or nonselective currents that are activated in parallel with a chloride current cannot be ruled out.

The increase in a current over time after patch rupture in whole-cell patch-clamp experiments is somewhat unusual. In general, currents diminish over time after establishing the whole-cell patch-clamp configuration. Two exceptions in cardiac cells are the delayed rectifier, when there is a low concentration of magnesium in the pipette solution,23 and the ATP-sensitive potassium current.24 The ATP-sensitive potassium current in the heart is considered to be of little functional significance in healthy myocardium, but its contribution to transmembrane potential increases under pathological conditions. By analogy, the time-dependent increase in chloride conductance in canine atrial cells, under the nonphysiological conditions imposed by whole-cell patch-clamp recording, suggests that chloride currents might contribute significantly to cardiac transmembrane potential under pathological conditions. Therefore, it becomes important to understand the mechanism responsible for the time-dependent activation of chloride current in canine atrial cells to determine if the stimulatory event(s) might occur in situ during pathology. Factors that might augment chloride current during

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**Figure 7.** Comparison of the change in whole-cell conductance observed during conventional whole-cell patch-clamp recording (ruptured patch) to that observed with perforated patch-clamp recording with nystatin. Whole-cell currents were normalized by cell capacitance and then averaged. Values plotted are mean ± SEM (ruptured patch, n=6; perforated patch, n=4). The dark lines are drawn through the average current values. The vertical lines represent the standard error at individual data points.
whole-cell patch-clamp include 1) cell swelling, 2) the washout or dilution of an endogenous inhibitor of the current, and 3) activation of the current by a component of the pipette solution.

Perforated patch recording was used to record from the cells with minimal disruption of the cytoplasm. The current did not spontaneously activate if perforated patch recording was used instead of ruptured patch recording. Cell swelling was initially considered an unlikely cause of current activation, because the intracellular and extracellular solutions used in the ruptured patch-clamp studies were isosmotic. However, the results of Worrell et al. suggested that the current might be activated by swelling, even though isosmotic solutions were being used. Careful monitoring of cell size demonstrated that there was indeed swelling (12% on average) during ruptured patch recordings. Swelling and chloride current were negated by hyperosmotic solutions. The results of the perforated patch recordings were also consistent with the hypothesis that the current observed during ruptured patch whole-cell recording was induced by cell swelling. No increase in chloride current was observed during nystatin experiments when the extracellular osmolarity was 293 mosm/kg. When swelling of atrial cells was induced by lowering extracellular osmolarity to 221 mosm/kg, chloride current was revealed. These results are consistent with the hypothesis, first stated by Worrell et al., that low mobility osmolytes within the cytoplasm are responsible for the phenomena observed. The low mobility osmolytes fail to equilibrate with the pipette solution after patch rupture and cause the cell interior to have a greater osmotic pressure than the pipette solution or the bath. Water can then enter the cell either from the pipette solution or from the extracellular solution. Cell swelling can then activate a chloride-sensitive conductance.

Cell swelling places stress on the cell membrane in whole-cell patch-clamp experiments for two reasons. First, there is the increase in wall tension that accompanies an increase in cell diameter. In whole-cell patch-clamp experiments, swelling will also induce stress at the junction between the electrode and the cell membrane. It cannot be concluded at this point whether stress on the membrane due to an increase in intracellular volume, stress at the junction between the microelectrode and the cell membrane, or both are responsible for the activation of this current.

In leukocytes (reviewed in Reference 25) and epithelial cells (reviewed in Reference 26), swelling-activated chloride currents have been identified. These currents are involved in regulatory volume decreases that can occur in the continuous presence of hypotonic extracellular solutions. The swelling-induced chloride current, in conjunction with a loss of counter ions, can decrease intracellular osmotic pressure and lead to loss of cellular water.
The swelling-induced chloride-sensitive current described in this article has the potential to contribute to a regulatory volume decrease in myocardial cells, but to date there are no reports to indicate that regulatory volume decreases occur in heart cells. A regulatory volume decrease was not observed in the cardiac myocytes used for this study. During whole-cell patch-clamp experiments, a regulatory volume decrease would not be expected; chloride and cation loss across the cell membrane could be replaced by the pipette solution. However, a regulatory volume decrease in cells that were not perturbed by a recording electrode might be expected to occur. To the contrary, no regulatory volume decrease was observed in undisturbed cells during the 5-minute exposures to hypotonic solutions used in this study. Likewise, no regulatory volume decrease was observed during 20-minute exposures to hypotonic solutions in a study by Drenowska and Baumgarten, who used rabbit cardiac myocytes.

Swelling and chloride-sensitive current activation were produced more readily in canine atrial cells than in canine ventricular cells, under the conditions used in the present study (cesium-containing solutions with no potassium). This explains why a spontaneously developing chloride current is observed during whole-cell patch-clamp recordings in canine atrial but not ventricular myocytes when identical solutions are used. It should be noted that a swelling-induced chloride-sensitive current can be observed in canine ventricular cells. However, a larger osmotic gradient is required to unmask the swelling-induced current in the ventricular cells compared with the atrial cells used for this study.

The results of this study do not imply that all atrial cells are more susceptible to hypotonic stress than ventricular myocytes. When exposed to saline solutions that are closer to being physiological than those used in this study, rabbit atrial and ventricular cells respond with identical width changes when the osmolarity of the bathing solutions is altered. The discrepancy between the present results and those of Clemo and Baumgarten can be attributed to differences in the species, the ionic conditions used, or artifacts induced by cell isolation.

The presence of a swelling-activated chloride current in cardiac muscle might have important implications for understanding pathophysiological events. Ischemia has been demonstrated to cause swelling of cardiac myocytes (reviewed in Reference 28); therefore, chloride currents might be unmasked during ischemia. The fact that the swelling-induced current is enhanced by isoproterenol is important because adrenergic tone is elevated during ischemia. The reversal potential for chloride is normally in the −50 mV range. Therefore, activation of a chloride current could have two effects that might...
contribute to abnormal electrical activity in the heart. At normal resting potentials, activation of a chloride current generates a depolarizing current that could lead to the initiation of abnormal automaticity. In the Harris dog (myocardial infarction created by a two-stage ligation of the left anterior descending artery), some of the arrhythmias present one day after infarction are considered to result from abnormal automatic activity in subendocardial Purkinje fibers (reviewed in Reference 29). Although some of the depolarization of subendocardial Purkinje fibers 24 hours after infarction can be explained by changes in the potassium equilibrium potential, a significant amount of depolarization cannot be accounted for by changes in the distribution of potassium ions. The second effect of chloride current activation that could contribute to arrhythmias is shortening of the action potential and refractory period. The action potential shortening could result from outward chloride current at plateau potentials. This would predispose the heart to reentrant arrhythmias. The possibility that chloride current might make a significant contribution to cardiac electrical activity under pathological conditions merits further evaluation.

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