Swelling-activated and Isoprenaline-activated Chloride Currents in Guinea Pig Cardiac Myocytes Have Distinct Electrophysiology and Pharmacology

JAMIE I. VANDENBERG, ATSUYA YOSHIDA, KIARAN KIRK, and TREVOR POWELL

From the University Laboratory of Physiology, Parks Road, Oxford, OX1 3PT, United Kingdom

ABSTRACT We have used the whole-cell patch clamp recording technique to characterize a swelling-activated chloride current in guinea pig atrial and ventricular myocytes and to compare the electrophysiological and pharmacological properties of this current with the isoprenaline-activated chloride current in the same cell types. Osmotic swelling of guinea pig cardiac myocytes caused activation of an outwardly rectifying, anion-selective current with a conductance and permeability sequence of \( I^- > NO_3^- > Br^- > Cl^- > Asp^- \). This current was inhibited by tamoxifen, 4,4'-diisothiocyanato-stilbene-2,2'-disulphonate and anthracene-9-carboxylic acid, in decreasing order of potency. The isoprenaline-activated anion current, like the swelling-activated current, had a higher permeability to \( I^- \) relative to \( Cl^- \), but it had a markedly reduced conductance for \( I^- \) compared to \( Cl^- \). The isoprenaline-activated current was insensitive to inhibition by tamoxifen, 4,4'-diisothiocyanato-stilbene-2,2'-disulphonate and anthracene-9-carboxylic acid. The swelling-activated current could be elicited in >90% atrial myocytes studied but only 34% ventricular myocytes. Conversely, the isoprenaline-activated current was elicited in <10% atrial myocytes and >90% ventricular myocytes. In those ventricular myocytes where it was possible to elicit swelling-activated and isoprenaline-activated currents simultaneously, the currents retained the same distinguishing characteristics as when they were elicited in isolation. Thus, while guinea pig atrial cells appear to preferentially express swelling-activated chloride channels and guinea pig ventricular myocytes preferentially express isoprenaline-activated chloride channels, the presence of these two channel types are not necessarily mutually exclusive. This raises the possibility that there may be coordinated regulation of the expression of different \( Cl^- \) channels within the heart.

Address correspondence to Jamie Vandenberg, University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, United Kingdom.

Dr. Yoshida's present address is Department of Anesthesiology, Faculty of Dentistry, Kyushu University, Fukuoka 812, Japan.
INTRODUCTION

A number of chloride-sensitive sarcolemmal conductances have been identified in cardiac myocytes (see reviews by Hume and Harvey, 1991; Ackerman and Clapham, 1993; Hwang and Gadsby, 1994). To date, the best characterized is the cAMP-activated chloride current ($I_{\text{Cl,cAMP}}$) which appears to be mediated by channels related to the cystic fibrosis transmembrane conductance regulator (CFTR; Nagel, Hwang, Nastiuk, Nairn, and Gadsby, 1992; Horowitz, Tsung, Hart, Levesque, and Hume, 1993). Other chloride conductances identified in cardiac tissue include a swelling-activated conductance ($I_{\text{Cl,swell}}$; Coulombe and Coraboeuf, 1992; Hagiwara, Masuda, Shoda, and Irisawa, 1992; Sorota, 1992; Tseng, 1992; Zhang, Rasmusson, Hall, and Lieberman, 1992, 1993), PKC-activated conductance ($I_{\text{Cl,PKC}}$; Walsh, 1991; Walsh and Long, 1994), Ca$^{2+}$-activated conductance ($I_{\text{Cl,ca}}$; Zygmunt and Gibbons, 1991) and purinergic-activated conductance ($I_{\text{Cl,p}}$; Matsuura and Ehara, 1992). Despite the significant progress in characterizing cardiac chloride currents, little is known of what biological roles they may subserve. $I_{\text{Cl,cAMP}}$ may be an important determinant of action potential duration when catecholamines are elevated (Levesque, Clark, Zaka-rov, Rosenstrauch, and Hume, 1993) and it has been suggested that $I_{\text{Cl,swell}}$ may contribute to regulatory volume decrease after cell swelling (Zhang et al., 1993); however, specific functions for the other currents remain to be determined. The potential importance of anion channels in arrhythmogenesis has been highlighted recently by experiments which have shown that perfusion of isolated rat hearts with solutions containing NO$_3^-$, in place of Cl$^-$, protects the heart against ischemia- and reperfusion-induced arrhythmias (Ridley and Curtis, 1992; Curtis, Garlick, and Ridley, 1993). Subsequently, Zhou and Lab (1994) have presented preliminary evidence suggesting that these effects may be related to activation of $I_{\text{Cl,swell}}$.

In this study, we undertook to characterize in more detail the electrophysiological and pharmacological properties of $I_{\text{Cl,swell}}$ in cardiac (guinea pig atrial and ventricu-lar) myocytes, using the whole-cell patch clamp technique. We have also compared the properties and distribution of $I_{\text{Cl,swell}}$ with $I_{\text{Cl,cAMP}}$ under the same experimental conditions in the same cell types.

MATERIALS AND METHODS

Myocyte Preparation

Single cardiac myocytes were dissociated from guinea pig hearts, obtained from animals killed by cervical dislocation after stunning, by digestion with collagenase and protease using established techniques described elsewhere (Powell, Terrar, and Twist, 1980; Mitchell, Powell, Terrar, and Twist, 1987). After the initial perfusion with the collagenase/protease digestion medium, the atria and ventricles were incubated separately and then digested as described previously. Isolated myocytes were stored at room temperature in Dulbecco's modified Eagle's medium (DMEM) solution, containing 25 mM HEPES and supplemented with 2% (vol/vol) of a serum substitute (Ultroser G, GIBCO, UK). Aliquots of cells were transferred to a bath on the stage of an inverted microscope (Nikon Diaphot) and superfused with Tyrode solution containing (in millimolar): NaCl, 140; KCl, 5.4; MgCl$_2$, 1.0; CaCl$_2$, 1.8; NaH$_2$PO$_4$, 0.33; glucose, 11; HEPES, 5 (pH adjusted to 7.4 at 22–24°C with NaOH). The experiments reported
here were carried out at 34–36°C (bath temperature was controlled via a thermocouple feedback circuit).

**Solutions**

The composition of the internal and external perfusion solutions were designed to block K⁺ and Ca²⁺ currents as well as electrogenic transporters. The isoosmotic external solution contained (in millimolar): NaCl, 140; MgCl₂, 2; BaCl₂, 2; HEPES, 5; and the pH was adjusted to 7.5 (at room temperature) with CsOH. Nicardipine, 2 μM, was added to block Ca²⁺ channels and ouabain, 20 μM, to block Na⁺-K⁺ pump current. In some experiments, 140 mM sucrose replaced 70 mM NaCl in the isoosmotic solution. The standard hyposmotic solution was as above except NaCl was reduced to 70 mM (or sucrose omitted). In the majority of experiments the internal (pipette) solution contained (in millimolar): CsCl, 58; CsAsp, 52; tetraethylammonium chloride, 20; EGTA, 10; MgATP, 5; Na₈GTP, 0.2; HEPES, 5; and the pH was adjusted to 7.3 (at room temperature) with CsOH or NaOH. In some experiments, the pipette [Cl⁻] was changed to 20 or 130 mM by isoosmotic replacement of CsCl with CsAsp or vice versa. In anion substitution experiments, the isoosmotic solutions contained 140 mM sucrose + 70 mM NaCl, NaI, NaNO₃, NaBr, or NaAsp. Hyposmotic solutions were as above except sucrose was removed. All anion substituted solutions therefore still contained 8 mM Cl⁻.

Unless otherwise stated in the text, cells were exposed to hyposmotic solutions for ≤5 min. Solution osmolalities were measured using a Roebling freezing point osmometer (Camlab, Cambridge, UK). The osmolality of the isoosmotic solutions were in the range 285–295 mosm kg⁻¹, hyposmotic solutions 155–165 mosm kg⁻¹ and internal solutions 280–290 mosm kg⁻¹.

Isoprenaline was prepared as a 1 mM stock solution, in water containing 100 mM ascorbic acid, and added to superfusion solutions to a final concentration of 1 μM.

**Electrophysiology**

The whole-cell tight-seal voltage clamp technique was used for electrophysiological recording (Hamill, Marty, Neher, Sakmann, and Sigworth, 1981). Patch electrodes were fabricated from glass capillaries (TW150-4, World Precision Instruments, Inc., USA) on a Narishige PP83 electrode puller (Narishige, Japan) and were used without fire polishing or coating with Sylgard®. To facilitate equilibration of the intracellular medium with the pipette solution, glass electrodes having large tip diameters were used (tip resistance ranged from 0.5–2 MΩ when filled with standard internal solution). After formation of a gigaohm seal, brief strong suction was applied to the pipette interior to rupture the membrane patch. After membrane rupture the suction port of the electrode holder was opened to the atmosphere to ensure that pressure was not applied to the back of the pipette.

Membrane current and voltage were recorded using a patch clamp amplifier (Axopatch 1C, Axon Instruments, USA). The current-voltage (I-V) relation was measured by applying a triangular ramp pulse of 1 V s⁻¹, first by depolarizing to either +110 or +70 mV followed by a hyperpolarization to −80 or −120 mV, respectively. The I-V curve was measured from the negative-going limb of the ramp pulse. Alternatively, whole cell currents were recorded during 300-ms rectangular pulses to potentials in the range −80 to +80 mV from a holding potential of 0 mV. These protocols were repeated at 5-s intervals, with both current and voltage monitored on an oscilloscope and chart recorder. The cell input capacitance was measured from the jump in membrane current recorded at the positive peak of the ramp pulse, and in the experiments reported here, typical input capacitance measured in a sample of ventricular cells was 124 ± 4 pF (mean ± SEM; n = 129) and in atrial cells was 30 ± 2 pF (n = 52). Current and voltage were digitized (CED 1401, Cambridge, UK) and then stored by computer (IBM-AT) for subsequent analysis. Analysis software was written by T. Shioya (Saga Medical
In all experiments, the patch pipette current was nulled before seal formation with the cells bathed in normal tyrode solution. In experiments where there was no anion substitution, a nonflowing 3-M KCl salt bridge between the Ag/AgCl reference electrode and bath solutions was used, with the 3-M KCI replaced each day. Anion substitution experiments (and measurements of liquid junction potentials, $V_{lj}$) were performed with the patch pipette containing 78 mM Cl$^-$ internal solution and a flowing 3-M KCl bridge between the Ag/AgCl reference electrode and the bath solutions (Neher, 1992). The silver reference electrode was rechlorided approximately every 6 h or whenever any drifts in zero-current potentials were observed. Changes in $V_{lj}$ after switches between normal tyrode and anion-substituted solutions stabilized after 20–30 s. $V_{lj}$ were therefore measured 45–60 s after switching between normal tyrode and anion-substituted solutions (and this same time period was used for the anion substitutions in the subsequent experiments). The liquid junction potentials were all < 1 mV except for the 70 mM NaAsp solutions ($V_{lj} = 3.5 \pm 0.3$ mV, $n = 3$). Individual records depicted in figures have not been corrected for junction potentials but all mean data (Table I) have been corrected.

Changes in conductance in the presence of inhibitors or after changes in external anions were measured from the $I$-$V$ curves obtained during voltage ramp experiments by linear least squares fit to the points ± 10 mV of the reversal potential ($E_{rev}$), as described in Lewis, Ross, and Cahalan (1993). In experiments where the swelling response had not reached a plateau, the conductance for chloride was calculated from the mean of the chloride conductances measured immediately before switching to the new anion solution and 30–40 s after returning to the chloride solution. The relative permeabilities of different anions were determined from the change in $E_{rev}$ after partial replacement of Cl$^-$ in the external solution with the test anion (as described in Overholt, Hobert, and Harvey, 1993). The shift in $E_{rev}$ ($\Delta E_{rev}$) was used to calculate the relative permeability of the replacement anion using the modified Goldman-Hodgkin Katz equation:

$$\Delta E_{rev} = \frac{RT}{F} \ln \left(\frac{[Cl]_c}{[Cl]_t + P_{rel}[A]_o}\right)$$

where $R$, $T$, and $F$ have their usual meanings; $P_{rel}$ (relative permeability = $P_A/P_{Cl}$), where $P_{Cl}$ and $P_A$ are the permeabilities of Cl$^-$ and the replacement anion, respectively; [Cl]$_c$ and [Cl]$_t$ are the concentrations of extracellular Cl$^-$ before and after replacement with the test anion respectively; and $[A]_o$ is the concentration of the replacement anion in the extracellular solutions.

Statistical Analysis

All results are reported as means ± SEM. Initial statistical comparisons within each experimental group were made using a one way analysis of variance. Subsequent comparisons of mean values within each group were tested using a t test (Armitage and Berry, 1987).

Photography

A Nikon F601M single-lens reflex camera was attached to the front port of the inverted microscope. To minimize the possibility that vibrations caused by opening of the camera shutter might damage the patch pipette seal, cells were lifted off the bath floor immediately after achieving the whole-cell patch configuration, by gently raising the patch electrode. Photographs were taken immediately after lifting the cell up, during cell swelling and after return to isoosmotic solution.
RESULTS

Effects of Anisosmotic Solutions on Atrial and Ventricular Myocytes

Superfusion of atrial and ventricular myocytes with hypoosmotic external solution resulted in cell swelling (see Fig. 1). In ventricular myocytes, changes in cell width were more marked than changes in cell length. The relative cell width changes in atrial myocytes were larger than those seen in ventricular cells and in the example shown in Fig. 1 there was also significant shortening of the cell during exposure to a hypoosmotic external solution. Return to isoosmotic external solution after brief periods (2–3 min) of superfusion with hypoosmotic solutions was associated with a return towards the preswelling cell dimensions (see photographs in row iv of Fig. 1). After more prolonged periods of cell swelling (5–15 min), blebs formed on the sarcolemmal membrane and eventually the cells lysed (presumably secondary to membrane rupture). In cells where bleb formation had occurred, return to isoosmotic solution was associated with either incomplete or no recovery towards control cell dimensions. Perfusion of ventricular myocytes with a hyperosmotic internal solution caused cells to swell with a time course similar to that evoked by an external hypoosmotic solution (data not shown).

Electrophysiological Effects of Cell Swelling on Cardiac Myocytes

The whole-cell current records shown in the bottom of Fig. 1 were obtained from the cells shown in the photographs in the top of the figure. In 34% of ventricular myocytes studied (144/422), swelling was associated with activation of a whole-cell current (e.g., see Fig. 2 a) that was outwardly rectifying and had a reversal potential similar to the chloride reversal potential ($E_{Cl}$, see below). In 61% of cells (257/422) there was no significant increase in whole-cell current (e.g., see Fig. 1 b) during exposure to hypoosmotic solution and in 5% of cells (21/422) there was a large increase in the whole cell current followed shortly thereafter by cell lysis. In almost all atrial myocytes studied (61/65) cell swelling was associated with an increase in whole cell current (Fig. 2 c) that had similar characteristics to that seen in 34% of ventricular myocytes. The swelling-activated current decreased on reperfusion of the cell with isoosmotic external solution (Fig. 2, a and c). Reversal, was usually complete after brief ($\leq 5$ min) exposure to hypoosmotic solution, however, after more prolonged periods (5–15 min), reversal was either incomplete (e.g., see Fig. 9) or there was no reversal with the cell lysing after return to isoosmotic external solution (e.g., see Fig. 8 a).

The properties of the swelling-activated current, outward rectification and reversal potential close to the theoretical $E_{Cl}$ (see below), are easily seen in voltage-ramp experiments such as those depicted in the bottom of Fig. 1. However, these results are only valid if the swelling-activated current is virtually time independent, i.e., it has rapid activation and deactivation kinetics and so the current changes seen as the voltage changes with time during the ramp are due to changes in the voltage and not to changes with time. Experiments where the current response to rectangular pulse voltage jumps was examined (Fig. 2) confirm that the swelling activated current in both ventricular (Fig. 2 a) and atrial (Fig. 2 b) myocytes does have very rapid kinetics. For comparison, the response of ventricular myocytes to superfusion with isoprena-
line, 1 µM, is illustrated in Fig. 2 c. The isoprenaline-activated current ($I_{cAMP}$) in ventricular myocytes, like the swelling-activated current, was time independent and had a reversal potential close to zero in the presence of symmetrical chloride. $I_{cAMP}$ was elicited in 75/81 ventricular myocytes, compared to being almost absent in atrial myocytes (1/14).

The $I-V$ plots shown in Fig. 2 suggest that $I_{swell}$ is outwardly rectifying in the presence of symmetrical chloride whereas $I_{cAMP}$ is approximately linear. The ratio of the membrane conductance measured at +40 and −40 mV during voltage ramp experiments in cells superfused with solutions containing 70 mM NaCl ± 140 mM sucrose confirmed this finding, i.e., the ratio was 1.81 ± 0.07 (n = 19) for $I_{swell}$ in ventricular cells and 1.78 ± 0.06 in atrial cells (n = 16) compared to 1.14 ± 0.03 (n = 19) for $I_{cAMP}$ in ventricular cells ($P < 0.05$ compared to $I_{swell}$ in ventricular cells).

**Chloride Selectivity of the Swelling-activated Current**

The reversal potentials for the swelling activated current measured in 86 ventricular myocytes are shown in Fig. 3. For the four different chloride gradients used in these experiments, the mean reversal potential was always similar to the predicted Cl$^-$ equilibrium potential, consistent with the current being carried by Cl$^-$ ions. Thus,
guinea pig ventricular myocytes appear to contain both $I_{C_{LAMP}}$ and a swelling-activated chloride current ($I_{C_{Lswell}}$). The reversal potential for the swelling-activated current in guinea pig atrial myocytes had a similar chloride sensitivity to that seen in ventricular myocytes. However, while a high percentage of ventricular myocytes appear to have $I_{C_{LAMP}}$ and only a low percentage of ventricular myocytes have $I_{C_{Lswell}}$, the converse is the case for atrial myocytes. This raises the question of whether the currents are mediated by a single-channel type that is differentially regulated in response to different stimuli (as has been suggested for $I_{C_{LAMP}}$, $I_{C_{Lswell}}$, and $I_{C_{LCa}}$ in epithelia; Kubitz, Warth, Allert, Kunzelmann, and Greger, 1992). The outward rectification of $I_{C_{Lswell}}$ compared to the almost linear $I-V$ curve for $I_{C_{LAMP}}$, in the presence of symmetrical chloride, however, suggests that these two currents are mediated by different channels. This issue was investigated further by comparing the anion selectivity and pharmacological properties of the two currents.

**FIGURE 1.**

- **a** ventricular myocyte
- **b** ventricular myocyte
- **c** atrial myocyte

- **(i)**
- **(ii)**
- **(iii)**
- **(iv)**

- **a** $-80 \text{ mV}$ $1 \text{ nA}$ $70 \text{ mV}$
- **b** $-80 \text{ mV}$ $1 \text{ nA}$ $70 \text{ mV}$
- **c** $-80 \text{ mV}$ $1 \text{ nA}$ $70 \text{ mV}$
 Ionic Selectivity of $I_{\text{Cl,swell}}$ and $I_{\text{Cl,AMP}}$ in Cardiac Myocytes

Under conditions of similar ionic strength (70 mM Na-anion ± 140 mM sucrose), both $I_{\text{Cl,swell}}$ and $I_{\text{Cl,AMP}}$ were sensitive to replacement of external Cl$^-$ with NO$_3^-$, I$^-$, Br$^-$ or Asp$^-$. The changes in slope conductance (measured at the reversal potential in the presence of each anion, see Methods) after anion replacement are summarized in Table I. The most marked difference between $I_{\text{Cl,swell}}$ and $I_{\text{Cl,AMP}}$ occurred with I$^-$ substitution. Replacement of external Cl$^-$ with I$^-$ resulted in an increase conductance of $I_{\text{Cl,swell}}$ (Fig. 4, a and c; ventricular and atrial myocytes, respectively) but a decreased conductance for $I_{\text{Cl,AMP}}$ (Fig. 4 b).

Anion permeabilities, relative to Cl$^-$, for $I_{\text{Cl,swell}}$ and $I_{\text{Cl,AMP}}$ were estimated from the shifts in reversal potential after partial replacement of external Cl$^-$ by the test anion. The sequence of anion permeabilities for both $I_{\text{Cl,swell}}$ and $I_{\text{Cl,AMP}}$ was I$^-$
**Pharmacological Inhibition of I_{Cl,well} and I_{CLAMP} in Cardiac Myocytes**

The stilbene derivative DIDS (1 mM) caused a rapid and complete inhibition of I_{Cl,well} in both ventricular (Fig. 5 a) and atrial (Fig. 6 a) myocytes. Reversal of the inhibition after washout of the DIDS was, however, slow and often incomplete (e.g., see Fig. 5 a). In some cells, DIDS caused a more rapid block of the current at positive membrane potentials and this inhibition persisted for longer than inhibition at negative potentials after washout of the drug (e.g., see Fig. 6 a). Lower concentrations of DIDS had a similarly rapid onset of action but produced less inhibition (0.1

\~\text{NO}_3^- > \text{Br}^- > \text{Cl}^- > \text{Asp}^-$. Thus, I$^-$ had an increased permeability but decreased conductance, compared to Cl$^-$, through I_{CLAMP} (see Fig. 4 b, Table I).
TABLE I

Relative Conductances ($g_s$), Shifts in Reversal Potential ($E_s$) and Permeabilities ($P_s$) for Different Anions Compared with Chloride ($E_{Cl}$)

| Anion | $g_s$ | $E_s$ - $E_{Cl}$ | $P_s$/$P_{Cl}$ | $g_s$ | $E_s$ - $E_{Cl}$ | $P_s$/$P_{Cl}$ | $g_s$ | $E_s$ - $E_{Cl}$ | $P_s$/$P_{Cl}$ |
|-------|-------|-----------------|--------------|-------|-----------------|--------------|-------|-----------------|--------------|
| I^-   | 5     | 1.19 ± 0.05     | -11 ± 0.5    | 6     | 1.13 ± 0.03     | -12 ± 1      | 9     | 0.42 ± 0.03     | -12 ± 1      |
| NO$_3^-$ | 4   | 1.08 ± 0.01     | -11 ± 2      | 4     | 1.12 ± 0.07     | -8 ± 1       | 4     | 1.12 ± 0.04     | -16 ± 2      |
| Br^-  | 4     | 0.96 ± 0.05     | -5 ± 0.05    | 4     | 1.08 ± 0.05     | -5 ± 0.3     | 4     | 1.02 ± 0.06     | -8 ± 2       |
| Cl^-  | 4     | 0.96 ± 0.05     | -5 ± 0.05    | 4     | 1.08 ± 0.05     | -5 ± 0.3     | 4     | 1.02 ± 0.06     | -8 ± 2       |
| Asp^- | 3     | 0.45 ± 0.04     | 46 ± 7       | 4     | 0.42 ± 0.05     | 43 ± 3       | 4     | 0.46 ± 0.02     | 51 ± 5       |

*Only experiments where the activation of $I_{Cl,swell}$ occurred within 2 min and the total duration of exposure to hypotonic solution lasted < 5 min are included in the table.

Conductances were determined from the slope of the $I$-$V$ curve measured over a ±10 mV range either side of the reversal potential for each anion and are expressed relative to that measured for chloride. Where the swelling response had not reached a plateau the conductance for chloride was calculated from the mean of the chloride conductances measured immediately before switching to the new anion solution and 30–40 s after returning to the chloride solution.

Relative permeabilities calculated using the Goldman-Hodgkin-Katz equation (see Materials and Methods). In all groups, the relative permeability sequence was I^- ~ NO$_3^-$ > Br^- > Cl^- > Asp^- (i.e., difference between I^- and NO$_3^-$ was not significant in any group).

*P < 0.05 compared to chloride within each group.
mM caused $61 \pm 9\%$ inhibition, $n = 4$, and $0.5\ mM$ caused $80\%$ inhibition, $n = 2$) which was associated with more rapid and complete reversal after washout of the drug (e.g., see Fig. 8 b, below). In contrast, $1\ mM$ DIDS had little effect on $I_{CL,AMP}$ (Fig. 6 b, and Fig. 8).

Anthracene-9-carboxylic acid (9-AC) was a less potent inhibitor than DIDS, of $I_{Cl,swell}$ and it had a less rapid onset of action (data not shown). After 2 min exposure to 9-AC, $1\ mM$, the slope conductance of $I_{Cl,swell}$ in ventricular myocytes was reduced by $51 \pm 7\%$ ($n = 6$) and in atrial myocytes by $55 \pm 7\%$ ($n = 3$). $I_{CL,AMP}$ was insensitive to inhibition by $1\ mM$ 9-AC (Fig. 7).

Tamoxifen, an anti-estrogen, has recently been identified as a potent inhibitor of $I_{Cl,swell}$ in epithelial cells (Valverde, Mintenig, and Sepulveda, 1993). We therefore tested its effect on $I_{Cl,swell}$ in guinea pig cardiac myocytes. Tamoxifen, unlike DIDS, had a delayed onset of action on $I_{Cl,swell}$; tamoxifen, $10\ \mu M$, caused a $34 \pm 6\%$ reduction in slope conductance, measured at the reversal potential, after $30\ s$ and a $72 \pm 9\%$ ($n = 8$) reduction after $2\ min$ in ventricular myocytes and a $30 \pm 2\%$ and $88 \pm 11\%$ ($n = 3$) reduction in atrial myocytes after $30\ s$ and $2\ min$, respectively. Furthermore, the reduction of current in the presence of tamoxifen was often biphasic (e.g., see Fig. 6 b). Prolonged exposure of swollen cells to tamoxifen ($10\ \mu M$ for $5-15\ min$) was associated with cell lysis. This effect of tamoxifen is not likely to be solely due to inhibition of the swelling-activated chloride channels as complete inhibition of these channels using DIDS, $1\ mM$, did not cause cell death (e.g., see Figs. 5 a and 6 a). The delayed onset of action and biphasic pattern suggest that the mechanism of action of tamoxifen may be different to that of DIDS. Tamoxifen was ineffective at inhibiting $I_{CL,AMP}$ (see Fig. 7).
FIGURE 6. Pharmacological sensitivity of $I_{cl,\text{swell}}$ in guinea-pig atrial myocytes. (a) Chart record of current responses to voltage ramps during cell swelling. The pipette solution contained 70 mM Cl$^-$. DIDS, 1 mM, caused a rapid and complete inhibition of $I_{cl,\text{swell}}$ (Inset) $I-V$ curve in control (○), after 110 s exposure to hypoosmotic solution (□, $E_{rev} = 0 \text{ mV}$) and 20 s after application of DIDS, 1 mM (■). This example shows that DIDS caused more rapid inhibition of $I_{cl,\text{swell}}$ at positive potentials. Conversely, after washout of the drug, there was more rapid recovery of the current at negative potentials compared with depolarized potentials. (b) Chart record of current responses to voltage ramps during cell swelling. The pipette solution contained 70 mM Cl$^-$. Tamoxifen, 10 μM, caused a progressive inhibition of $I_{cl,\text{swell}}$ (Inset) $I-V$ curve in control (○), after 130 s exposure to hypoosmotic solution (●, $E_{rev} = -8 \text{ mV}$), 30 s after application of Tamoxifen, 10 μM (●, 29% reduction in slope conductance at the reversal potential) and 2 min after application of Tamoxifen, 10 μM (■, 90% reduction in slope conductance at the reversal potential). These examples also illustrate that atrial cells in general were able to tolerate longer periods of exposure to swelling compared to ventricular cells and more frequently showed full recovery back to the control values after reversal of cell swelling.

FIGURE 7. Summary of the effects of DIDS (1 mM), 9-AC (1 mM) and tamoxifen (10 μM) on $I_{cl,\text{swell}}$ in ventricular and atrial myocytes, and $I_{cl,\text{clamp}}$ in ventricular myocytes. Inhibition was measured as percent of reduction in the slope conductance of the current at the reversal potential (see Materials and Methods). Numbers in parentheses above each bar indicate the number of determinations for each value (mean ± SEM). DIDS (1 mM) caused 100% inhibition of $I_{cl,\text{swell}}$ in both ventricular and atrial myocytes but only a 12% reduction of $I_{cl,\text{clamp}}$. 9-AC (1 mM) caused a 51 and 55% reduction in $I_{cl,\text{swell}}$ in ventricular and atrial myocytes, respectively, but only a 7% reduction in $I_{cl,\text{clamp}}$. Tamoxifen (10 μM, after 2 min exposure) caused a 72 and 88% reduction in $I_{cl,\text{swell}}$ in ventricular and atrial myocytes respectively but only an 8% reduction in $I_{cl,\text{clamp}}$. 
**IClAMP and IClBulk May Be Elicited in the Same Ventricular Myocyte**

In a series of experiments, cells were first superfused with hypoosmotic solution and in those cells where a swelling-activated current was observed the cells were subsequently exposed to isoprenaline, 1 μM. In the example illustrated in Fig. 8a, the

μM caused a rapid increase in the whole-cell current. This current decayed slowly after withdrawal of isoprenaline. This cell died shortly after the end of the trace shown. (b) Difference I-V curves from the current traces indicated in the chart record shown in a. The reversal potential of the swelling-activated current (■△) was 1 mV (Ecl = -3 mV) and the current was outwardly rectifying. The reversal potential for the isoprenaline-activated current (●○) was 2 mV (Ecl = -3 mV) and the current had a linear slope. (c) Chart record of whole cell current responses to voltage ramps during exposure to isoprenaline (1 μM) and subsequent exposure to hypoosmotic external solution. Isoprenaline caused a rapid and sustained increase in the whole-cell current. Exposure to hypoosmotic external solution in the continued presence of isoprenaline caused a further gradual increase in the whole-cell current, after a delay of ~25 s. This current increase was reversible after return to isoosmotic solution. A subsequent exposure to hypoosmotic solution (in the continued presence of isoprenaline) caused an increase in the whole-cell current with a similar time course of activation to the first episode. In this second episode, the increased whole-cell current was rapidly and completely reversed by DIDS, 0.5 mM. After withdrawal of isoprenaline, there was a slow decline in the whole-cell current towards the initial level. Comparison of the trace in c with the trace in a also illustrates that while ventricular myocytes could withstand prolonged exposure to isoprenaline (5–15 min) and short exposures (2–3 min) to hypoosmotic external solution, prolonged exposure to hypoosmotic solution (5–20 min) was often associated with either incomplete recovery of the whole cell current to preswelling levels or with cell lysis. (d) Difference currents for the isoprenaline-activated current (●○, Erev = -22 mV, Ecl = -20 mV) and the swelling-activated current (■△, Erev = -20 mV, Ecl = -3 mV) for the cell illustrated in c.

Current elicited by cell swelling (in the presence of symmetrical chloride, [Cl⁻]i = [Cl⁻]pip = 78 mM) had a reversal potential of +1 mV and was outwardly rectifying (see Fig. 8b). This current was also sensitive to inhibition by DIDS. In the continuing presence of hypoosmotic external solution containing 0.5 mM DIDS, a
whole-cell current was elicited by superfusion of the cell with isoprenaline, 1 μM, that had a reversal potential of +2 mV and an approximately linear \( I-V \) curve (see Fig. 8b), i.e., similar to the properties of the isoprenaline-activated current elicited in the absence of cell swelling (e.g., see Fig. 5b). In embryonic chick heart cells, it has been found that cAMP inhibits \( I_{\text{Cl,sell}} \) (Hall, Zhang, and Lieberman, 1993). We therefore investigated whether exposure of cells to isoprenaline, before cell swelling, prevented activation of \( I_{\text{Cl,sell}} \) in guinea pig cardiac myocytes. In the example illustrated in Fig. 8c, exposure to isoprenaline, 1 μM, was associated with an increase in the whole-cell current that reached a new steady state (similar to that observed previously, e.g., compare with Fig. 5b). Subsequent exposure to hypoosmotic external solution was associated with a further increase in the whole-cell current that was sensitive to DIDS, 0.5 mM. In 16 cells first exposed to isoprenaline (1 μM, for 1 min), subsequent cell swelling was associated with activation of \( I_{\text{Cl,sell}} \) in five cells (i.e., 31%, a similar percentage to that seen in cells not first exposed to isoprenaline, 34%).

In atrial myocytes, as in ventricular myocytes, exposure to isoprenaline did not prevent activation of \( I_{\text{Cl,sell}} \) (see Fig. 9). Similar results to that illustrated in Fig. 9 were obtained in five out of six atrial cells. In the remaining cell pretreated with isoprenaline there was no activation of \( I_{\text{Cl,sell}} \) during exposure to hypoosmotic external solution.

**DISCUSSION**

When whole-cell patch clamped ventricular myocytes were exposed to a hypoosmotic external solution (~45% reduction in osmolality) the cell width increased significantly without any significant change in cell length (see Fig. 1). This finding is consistent with those reported previously (Roos, 1986; Drewnowska and Baumgarten, 1991). Under the conditions of whole-cell patch clamp recordings the relatively small intracellular volume of a cardiac myocyte (~20 pl, Nash, Tatham, Powell, Twist, Speller, and Loverock, 1979) is in continuity with a much larger patch pipette volume (~50 μl). It is therefore not surprising that we were not able to observe regulatory volume changes nor able to accurately correlate the extent of swelling with the size of...
the osmotic gradient across the cell membrane (for further discussion of this point see Worrell, Butt, Cliff, and Frizzell, 1989; and Doroshenko and Neher, 1992).

Under conditions where K+ channels, Ca2+ channels and electrogenic transporters were blocked, cell swelling in guinea pig ventricular and atrial myocytes was associated with activation of a current that was time independent (Fig. 2), outwardly rectifying (e.g., see Figs. 1, a and c and 2, a and b) and had a reversal potential similar to the equilibrium potential for chloride (Fig. 3). Furthermore, there was always a delay, of between 30-180 s after switching to the hypoosmotic external solution, before the swelling-activated current was observed. These characteristics are very similar to those reported for Icl,swell in many tissue types including lymphocytes (Lewis et al., 1993), neutrophils, (Stoddard, Steinbach, and Simchowitz, 1993), chromaffin cells (Doroshenko and Neher, 1992), epididymal cells (Chan, Fu, Chung, Huang, Zhou, and Wong, 1992), endothelial cells (Nilius, Oike, Zahradnik, and Droogmans, 1994), and a number of epithelial cell lines (e.g., Worrell et al., 1989; Diaz, Valverde, Higgins, Rucareanu, and Sepulveda, 1993; Rasola, Galietta, Gruernert, and Romeo, 1993; Valverde et al., 1993) and cardiac cells (Hagiwara et al., 1992, Sorota, 1992, Tseng, 1992, Zhang et al., 1993). It has previously been reported that guinea pig ventricular myocytes do not contain Icl,swell (Walsh and Long, 1994), however, only a small number of cells were investigated in that study. It is worth noting that a similar situation occurred with canine ventricular myocytes where Sorota (1992) suggested that only atrial and not ventricular myocytes contained Icl,swell but Tseng (1992), in a more comprehensive investigation, found that canine ventricular myocytes also contain Icl,swell.

The anion permeability sequence for Icl,swell is generally SCN- > I- > NO3 > Br- > Cl- > F- > Asp- (e.g., see Rasola, et al. 1993 and references therein; Lewis et al., 1993). Our results with guinea pig ventricular and atrial myocytes are consistent with this sequence. Also of note is that Asp- has a moderate conductance relative to chloride (see Table I; also see e.g., Banderali and Roy, 1992; Jackson and Strange, 1993; Tseng, 1992) and this may have significant physiological relevance for volume-regulatory amino acid fluxes (see below).

Icl,swell in guinea pig cardiac myocytes is sensitive to inhibition by DIDS (see Figs. 5 a and 6 a). Significant inhibition required high concentrations of DIDS (0.1–1 mM), however, it had a rapid onset of action. Furthermore, in some cells, DIDS was more effective at depolarized potentials (compare, e.g., with Chan et al., 1993; Doroshenko and Neher, 1992; Diaz et al., 1993; Hagiwara et al., 1992; Lewis et al., 1993). Recently, tamoxifen, an anti-estrogen, was found to be the most potent blocker, so far identified, of Icl,swell (in intestinal T84 cells, Valverde et al., 1993). This is similar to the findings reported here in guinea pig ventricular and atrial myocytes, where tamoxifen, 10 μM, was able to cause >70% inhibition within 2 min of exposure. This relatively slow onset of action of tamoxifen was in marked contrast to the rapid onset of action of DIDS (see Fig. 6). Possible explanations for this difference may be that tamoxifen acts from the intracellular surface (and hence the delay is due to the time taken to diffuse into the cell) or it may be working by affecting a regulation or signaling process (either within the cytoplasm or in the cell membrane). In previous studies of Icl,swell in cardiac tissue, 1 mM 9-AC was found to cause significant inhibition of the current. Thus, for example, Tseng (1992) found a 70–80% decrease
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in \( I_{Cl,swell} \) in canine ventricular myocytes after 8–10 min exposure to 9-AC, 1 mM. Both the extent of inhibition and the delayed onset of action were similar to what we observed in guinea pig cardiac myocytes in this study.

The best characterized Cl\(^-\) current in cardiac myocytes is \( I_{Cl,AMP} \). The electrophysiological properties of \( I_{Cl,AMP} \) reported here are broadly consistent with the previous studies: i.e., time-independent, almost-linear \( I-V \) relation in symmetrical chloride, and anion conductance and permeability sequence of NO\(^3^-\) > Br\(^-\) > Cl\(^-\) > Asp\(^-\) (see Hume and Harvey, 1991; Ackerman and Clapham, 1993; and Hwang and Gadsby, 1994, for reviews). There are, however, conflicting results in previous studies of I\(^-\) permeability in cardiac cells, e.g., Overholt et al. (1993) noted a decreased permeability for I\(^-\) compared to Cl\(^-\) but Walsh and Long (1992) and Dousmanis and Gadsby (1994) reported an increased permeability for I\(^-\) compared to Cl\(^-\). Our findings of a decreased conductance but increased permeability for I\(^-\) compared to Cl\(^-\) therefore support the findings of the latter studies.

The lack of pharmacological inhibitors of \( I_{Cl,AMP} \) reported here (e.g., see Figs. 5b and 7) is also reviewed in Hwang and Gadsby (1994). Levesque et al. (1993), however, found that 9-AC, 200 \( \mu \)M caused significant inhibition of \( I_{Cl,AMP} \), which contrasts with the < 10% inhibition seen in our study with 9-AC, 1 mM. In our study, the inhibitors were applied for 2 min so we cannot exclude the possibility that a more prolonged exposure might have produced some inhibition of \( I_{Cl,AMP} \). The lack of efficacy of tamoxifen and DIDS as inhibitors of \( I_{Cl,AMP} \) compared to their effects on \( I_{Cl,swell} \) suggests that under conditions where both \( I_{Cl,AMP} \) and \( I_{Cl,swell} \) may be activated, for example during myocardial ischemia, either DIDS (e.g., see Fig. 8) or tamoxifen may be useful pharmacological tools for separating out the relative contributions of each current.

Most workers believe that distinct channels are responsible for \( I_{Cl,swell} \), \( I_{Cl,AMP} \), and \( I_{Cl,Ca} \) (e.g., Valverde et al., 1993), however, it has been suggested that they may represent differential regulation of a single-channel type (Kubitz et al., 1992). In guinea pig cardiac myocytes, \( I_{Cl,AMP} \) and \( I_{Cl,swell} \) had different electrophysiological, anion selectivity, and pharmacological characteristics suggesting that they are mediated by distinct channel types. Furthermore, when the two currents were elicited in the same cell, the differences remain (e.g., linear versus outwardly rectifying \( I-V \) curves and sensitivity to DIDS, see Fig. 8). In the later experiments, it was also noted that cAMP did not appear to inhibit activation of \( I_{Cl,swell} \) in contrast to the findings reported by Hall et al. (1993). The possibility that \( I_{Cl,swell} \) may be regulated differently in different species or at different stages during development may explain this discrepancy (e.g., see Coulombe and Coraboeuf, 1992).

**Distribution of Chloride Channels in the Heart**

We have no explanation, as yet, for the finding that \( I_{Cl,swell} \) could only be elicited in one third of ventricular myocytes. It is possible that the channels may only be expressed in one third of the cells or that all myocytes contain \( I_{Cl,swell} \) channels but only some express a regulatory protein such as \( pI_{Cl,n} \), that has been shown to be required for activation of \( I_{Cl,swell} \) in some tissue types (Ackerman, Wickman, and Clapham, 1994). Another possibility is that there is a regulatory factor that has not been controlled for in our study or that the low percentage of apparent expression may be an artefact of the cell preparation, e.g., during the collagenase/protease
perfusion, a critical extracellular domain of the channels may be cleaved, although the finding that the channel appears to be expressed in nearly 100% of atrial myocytes would argue against a nonspecific artefact of the cell preparation procedure. Finally, it is possible that the variable delay between the onset of the response and the lack of response in some cells may reflect differences in the rate and/or extent of cell swelling or membrane stretch in different cells. In support of this contention, atrial cells appeared to swell more than ventricular cells (see Fig. 1) and had a higher percentage response. However, in six ventricular myocytes exposed to hypoosmotic solution for an extended period (10–20 min, i.e., until cell lysis occurred) there was still no activation of \( I_{\text{Cl,swell}} \). Nevertheless, as it was not possible to measure the exact cell volume or rate of volume increase in our experiments we cannot state definitively whether this can explain why activation of \( I_{\text{Cl,swell}} \) was observed in only 34% of ventricular myocytes. There are, however, precedents for Cl− channels being observed in only a subset of cardiac myocytes, for example, \( I_{\text{Cl,PKC}} \) was seen in only ~50% of guinea pig ventricular myocytes (Walsh and Long, 1994) and \( I_{\text{Cl,P}} \) in ~50% guinea pig atrial myocytes (Matsuura and Ehara, 1992). Thus, it would seem that the simplest explanation for our results is that \( I_{\text{Cl,swell}} \) is expressed in only a subset of guinea pig ventricular myocytes. The reason(s) for the apparent subset expression of \( I_{\text{Cl,swell}}, I_{\text{Cl,P}}, \) and \( I_{\text{Cl,PKC}} \) remain to be determined.

Previous work suggested that cardiac myocytes which contain \( I_{\text{Cl,swell}} \) do not contain \( I_{\text{Cl,CAMP}} \) (compare Hagiwara et al., 1992; Sorota, 1992; and Tseng, 1992, with e.g., Takano and Noma, 1992; and Collier, Levesque, Hart, Geary, Torihashi, Horowitz, and Hume, 1994). The results presented here (see Fig. 8), however, indicate that expression of \( I_{\text{Cl,CAMP}} \) and \( I_{\text{Cl,swell}} \) are not necessarily mutually exclusive. A similar complementary distribution of \( I_{\text{Cl,CAMP}} \) and \( I_{\text{Cl,swell}} \) has been suggested to occur in other tissues, for example in intestinal epithelium, Trezise and colleagues (Trezise, Romano, Gill, Hyde, Sepulveda, Buchwald, and Higgins, 1992) have shown that as cells migrate across the crypt-villus boundary, there is a decrease in expression of CFTR while expression of p-glycoprotein (a protein that may be associated with \( I_{\text{Cl,swell}} \) activity) increases. These results raise the possibility that there may be coordinated regulation of the expression of different Cl− channels at the organ level.

**Physiological Significance of \( I_{\text{Cl,swell}} \) in Cardiac Myocytes**

Activation of channels and pumps by changes in cell volume does not necessarily mean that they will participate in volume regulation (see review by Parker, 1993). There is good evidence for the involvement of the Na+–K+–2 Cl− transporter regulating cell volume after shrinkage (Drewnowska and Baumgarten, 1991; Clemo and Baumgarten, 1991) and more recently, Zhang et al. (1993) have shown that regulatory volume decrease is inhibited by chloride depletion although the pathway that contributes to this was not definitively characterized. It has been suggested that swelling-activated Cl− channels might provide a route for the volume regulatory efflux of amino acids after cell swelling (see e.g., Banderali and Roy, 1992; Kirk, Ellory, and Young, 1992; Kirk and Kirk, 1993; Jackson and Strange, 1993). However, whether the volume regulatory release of amino acids in cardiac cells (as, e.g., has been shown by Rasmusson, Davis, and Lieberman, 1993) is mediated by swelling-activated chloride channels remains to be determined.
Ventricular Arrhythmias

The reversal potential for chloride in cardiac myocytes is \( \sim -48 \text{ mV} \) (for \([\text{Cl}^-]_o = 120 \text{ mM}\) and \([\text{Cl}^-]_i = 20 \text{ mM}\), respectively; Vaughan-Jones, 1979). Therefore, chloride currents will produce inward (depolarizing) current at the normal resting membrane potential but outward (repolarizing) currents during the plateau of the action potential. In a series of studies, it has been reported that replacement of extracellular Cl\(^-\) with NO\(_3^-\) decreases the incidence of ischemia- and reperfusion-induced ventricular fibrillation in Langendorff-perfused rat hearts (Ridley and Curtis, 1992; Curtis et al., 1993). It is well known that myocardial cells swell during ischemia (Jennings, Reimer, and Steenbergen, 1986) and that after reperfusion, the washout of hypotonic extracellular fluid is likely to contribute to further cell swelling. Recently, Zhou et al. (1994) have shown that stretch-induced arrhythmias in guinea pig hearts are similarly reduced by replacement of extracellular Cl\(^-\) with NO\(_3^-\), thus suggesting that the mechanism underlying the observations of Ridley and Curtis (1992) and Curtis et al. (1993) may involve \( I_{\text{Cl,swell}} \). If this were to be the case, then our findings that NO\(_3^-\) has a higher permeability and conductance than Cl\(^-\) through \( I_{\text{Cl,swell}} \) would imply that the beneficial action of NO\(_3^-\) may be due to augmentation of \( I_{\text{Cl,swell}} \). Alternatively, the replacement of external Cl\(^-\) with the more permeant NO\(_3^-\) will, at least temporarily, result in an effective negative shift of the reversal potential (see Table I) and so its beneficial action may be to lessen depolarization of the resting membrane potential. These hypotheses, however, remain to be tested directly.

We gratefully acknowledge the technical assistance of Victor Twist.

This work was funded partly by a Program Grant from the Medical Research Council and partly by a Project Grant from the British Heart Foundation. J. I. Vandenberg received support from the Sydney University Medical Foundation, the British Heart Foundation, and is a Zeneca Junior Research Fellow in Medical Sciences (Pembroke College, Oxford). A. Yoshida received support from the Wellcome Trust. K. Kirk is a Lister Institute Research Fellow and Staines Medical Research Fellow (Exeter College, Oxford)

Original version received 16 June 1994 and accepted version received 12 September 1994.

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