Swelling-activated Gd\(^{3+}\)-sensitive Cation Current and Cell Volume Regulation in Rabbit Ventricular Myocytes

HENRY F. CLEMO\(^*\) and CLIVE M. BAUMGARTEN\(^\dagger\)

From the \(^*\)Department of Internal Medicine (Cardiology), and \(^\dagger\)Department of Physiology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298

ABSTRACT The role of swelling-activated currents in cell volume regulation is unclear. Currents elicited by swelling rabbit ventricular myocytes in solutions with 0.6–0.9× normal osmolarity were studied using amphotericin perforated patch clamp techniques, and cell volume was examined concurrently by digital video microscopy. Graded swelling caused graded activation of an inwardly rectifying, time-independent cation current (I\(_{\text{Cir,swell}}\)) that was reversibly blocked by Gd\(^{3+}\), but I\(_{\text{Cl,swell}}\) was not detected in isotonic or hypertonic media. This current was not related to I\(_{\text{K1}}\) because it was insensitive to Ba\(^{2+}\). The P\(_{\text{K}}\)/P\(_{\text{Na}}\) ratio for I\(_{\text{Cir,swell}}\) was 5.9 ± 0.3, implying that inward current is largely Na\(^+\) under physiological conditions. Increasing bath K\(^+\) increased g\(_{\text{Cir,swell}}\) but decreased rectification. Gd\(^{3+}\) block was fitted with a K\(_{0.5}\) of 1.7 ± 0.3 μM and Hill coefficient, n, of 1.7 ± 0.4. Exposure to Gd\(^{3+}\) also reduced hypotonic swelling by up to ~30%, and block of current preceded the volume change by ~1 min. Gd\(^{3+}\)-induced cell shrinkage was proportional to I\(_{\text{Cir,swell}}\) when I\(_{\text{Cir,swell}}\) was varied by graded swelling or Gd\(^{3+}\) concentration and was voltage dependent, reflecting the voltage dependence of I\(_{\text{Cir,swell}}\). Integrating the blocked ion flux and calculating the resulting change in osmolarity suggested that I\(_{\text{Cir,swell}}\) was sufficient to explain the majority of the volume change at ~80 mV. In addition, swelling activated an outwardly rectifying Cl\(^-\) current, I\(_{\text{Cl,swell}}\). This current was absent after Cl\(^-\) replacement, reversed at E\(_{\text{Cl}}\), and was blocked by 1 mM 9-anthracene carboxylic acid. Block of I\(_{\text{Cir,swell}}\) provoked a 28% increase in swelling in hypertonic media. Thus, both cation and anion swelling-activated currents modulated the volume of ventricular myocytes. Besides its effects on cell volume, I\(_{\text{Cir,swell}}\) is expected to cause diastolic depolarization. Activation of I\(_{\text{Cir,swell}}\) also is likely to affect contraction and other physiological processes in myocytes.

KEY WORDS: osmoregulation • stretch-activated channels • mechano-electrical feedback • arrhythmias

INTRODUCTION

Ion channels activated by mechanical stretch or cell swelling (SAC)\(^1\) are present in cells ranging in complexity from primitive unicellular organisms to highly differentiated neurons and myocytes (Sachs, 1988; Morris, 1990; Sackin, 1995; Sukharev et al., 1996; Vandenberg et al., 1996). Members of this class of channels exhibit varying selectivity, conductance, kinetics, and requirements for activation. In cardiac myocytes, for example, osmotic or hydrostatic pressure-induced cell swelling activates Cl\(^-\) channels (Tseng, 1992; Sorota, 1992; Hagiwara et al., 1992; Coulombe and Coraboeuf, 1992; Zhang et al., 1993), and mechanical deformation or swelling activate both poorly selective cation (Craeft, 1988; Bustamante et al., 1991; Sigurdson et al., 1992; Sadoshima et al., 1992; Kim, 1993; Kim and Fu, 1993; Ruknudin et al., 1993) and K\(^+\)-selective channels (Brezden et al., 1986; Kim, 1992; Sasaki et al., 1992; Van Wagoner, 1993; Ruknudin et al., 1993).

The broad distribution of SACs suggests their sensitivity to mechanical stretch or swelling must subserve fundamental cellular functions or reflect fundamental properties of ion channels. One appealing physiological role is in cell volume regulation. After rapid swelling on exposure to hypotonic media, activation of transport pathways allows many cell lines to normalize their volume in a process called a regulatory volume decrease (RVD). SACs have been implicated in the RVD observed in Ehrlich ascites tumor cells (Lambert and Hoffman, 1994), renal cortical duct cells (Schwiebert et al., 1994), and embryonic chick cardiac myocytes (Rasmussen et al., 1993; Zhang et al., 1993). In contrast, rabbit myocytes can swell significantly without an RVD (Drewnowska and Baumgarten, 1991; Suleymanian and Baumgarten, 1996).

To investigate whether SACs regulate cell volume in mammalian cardiac myocytes, Suleymanian et al. (1995) used Gd\(^{3+}\) and 9-anthracene carboxylic acid (9-AC) as markers of channel activity. Gd\(^{3+}\) blocks stretch-activated, poorly selective cation channels (Yang and Sachs, 1989; Sadoshima et al., 1992; Hamill and McBride, 1996), and 9-AC blocks swelling-activated Cl\(^-\) channels.
(Tseng, 1992; Sorota, 1992, 1994; Hagiwara, 1992; Vandenberg et al., 1994). Under physiological osmotic conditions, when SACs are presumed to be silent, neither blocker alters the volume of isolated rabbit ventricular cells. In hypotonic solutions, however, Gd³⁺ significantly decreases and 9-AC significantly increases rabbit ventricular myocyte volume (Suleymanian et al., 1995). These data on intact, unclamped cells were explained by suggesting that osmotic swelling activates Gd³⁺- and 9-AC-sensitive ion channels that mediate sustained cation and anion fluxes, respectively, and in turn modulate cell volume.

Volume regulation in cultured chick myocytes appears to be different than in freshly isolated rabbit myocytes. Chick heart cells exhibit a strong RVD in response to osmotic swelling that is attenuated by removing Cl⁻ (Rasmussen et al., 1993). Osmotic and hydrostatic swelling is sustained and activates a Gd³⁺-sensitive Cl⁻ current during ruptured patch whole-cell recordings (Zhang et al., 1993; Zhang and Lieberman, 1996). These workers concluded, however, that the swelling-induced Cl⁻ current does not contribute to regulation of chick heart cell volume because a complete RVD occurred and a Cl⁻ current was not generated under perforated patch conditions (Hall et al., 1995). This emphasizes that ruptured patch techniques may distort cell volume regulation.

The present work describes a swelling-induced, Gd³⁺-sensitive current in rabbit ventricular myocytes and examines its role in cell volume regulation. The perforated patch voltage-clamp technique and video microscopy were used to simultaneously determine whole-cell ionic currents and the relative cell volume in the absence and presence of osmotic swelling. The experiments demonstrated that; (a) osmotic swelling elicited a graded, inwardly rectifying, Gd³⁺-sensitive cation current, termed Iₘ,swell, that could be separated from an outwardly rectifying, 9-AC-sensitive anion current, I₃₉,swell; (b) Iₘ,swell poorly distinguishes between K⁺ and Na⁺; (c) block of Iₘ,swell by Gd³⁺ reduces the volume of osmotically swollen cells in a swelling- and voltage-dependent manner; and (d) the magnitude of the Gd³⁺-sensitive current can largely account for the Gd³⁺-induced cell shrinkage. A preliminary report appeared previously (Clemo and Baumgarten, 1995).

**Materials and Methods**

**Cell Isolation**

Ventricular myocytes were freshly isolated from New Zealand white rabbits (2.5–3 kg) using a collagenase–pronase dispersion method as previously described (Clemo and Baumgarten, 1991; Clemo et al., 1992). Cells were stored in a modified Kraft-Brühe solution containing (mM): 132 KOH, 120 glutamic acid, 2.5 KCl, 10 KH₂PO₄, 1.8 MgSO₄, 0.5 K₃EGTA, 11 glucose, 10 taurine, 10 HEPES (pH 7.2, 295 mosm/liter). Typical yields were 50–70% Ca²⁺-tolerant, rod-shaped cells. Myocytes were used within 6 h of harvesting, and only quiescent cells with no evidence of membrane blebbing were selected for study.

**Experimental Solutions**

Cells were placed in a glass-bottomed chamber (~0.3 ml) and superfused with room temperature (21–22°C) bathing solution at 3 ml/min. Solution changes were complete within 10 s, as estimated from the liquid junction potential of a microelectrode. The standard bathing solution contained (mM): 65 NaCl, 5 KCl, 2.5 CaSO₄, 0.5 MgSO₄, 5 HEPES, 10 glucose, and 17–283 mannitol (pH 7.4). The reduced, fixed NaCl concentration permitted adjustment of osmolarity with mannitol at a constant ionic strength. An osmolarity of 296 mosm/liter was taken as isotonicity (1T). Osmolarity ranged from 178 to 266 mosm/liter in hypotonic solutions (0.6T–0.9T) and was 444 mosm/liter in hypertonic solution (1.5T). Osmolarity routinely was verified with a freezing-point depression osmometer (Osmette S; Precision Systems Inc., Natick, MA).

To evaluate the roles of specific ions, nominally Ca²⁺-free, Na⁺-, K⁺-, and Ca²⁺-, free, and Cl⁻- and Ca²⁺-free solutions were prepared. MgSO₄ replaced CaSO₄, Nmethyl-d-glucamine (NMDG) Cl replaced NaCl and KCl, or Na and K methanesulphonate replaced NaCl and KCl on equimolar bases.

**Voltage Clamp**

Electrodes were pulled from 7740 glass capillary tubing (1.5 mm o.d., 1.12 mm i.d., filamente; Glass Co. of America, Bargaintown, NJ) to give a final tip diameter of 3–4 μm and a resistance of 0.5–1 MΩ. The standard electrode filling solution contained (mM): 120 K aspartate, 10 KCl, 3 MgSO₄, 10 HEPES, pH 7.1. In addition, a Na⁺- and K⁺-free pipette solution was made by replacing Na⁺ and K⁺ salts with Cs⁺ salts, and a low Cl⁻ (5 mM) pipette solution was made by replacing NaCl and KCl with the corresponding aspartate salts.

Whole-cell currents were measured using an Axoclamp 200A amplifier (Axon Instruments, Inc., Foster City, CA). Pulse and ramp protocols, voltage-clamp data acquisition, and offline data analysis were controlled with custom programs written in ASSIST (Keithley, Taunton, MA). Both step and ramp voltage-clamp protocols were applied (see Fig. 1, D and G), and holding potential (Eₜ₉) was either −80, −40, or 0 mV. In step protocols, the test voltage step was 500 ms in duration. Current during the last 50 ms of the step was averaged and called steady state current. In ramp protocols, the voltage was stepped from Eₜ₉ to +40 mV for 20 ms, ramped to −100 mV over 5 s, and, after 10 ms, ramped back to +40 mV over 5 s. To cancel the capacitive current, the depolarizing and hyperpolarizing arms of the ramp were averaged. A very slow voltage ramp (28 mV/s) was used so as to approach steady state, and ramp current–voltage (I-V) relationships were in good agreement with those obtained by voltage steps. Nevertheless, the contribution of slowly activating currents such as the slow component of the delayed rectifier, Iₐ, may be underestimated. Both step and ramp currents were digitized at 1 kHz and low-pass filtered at 200 Hz. The reported Eₜ₉ was corrected for the liquid junction potential of the patch electrode as measured from the change in potential upon switching the bath between the superfusing and electrode-filling solutions (Nehér, 1992), and the bath was grounded with a 3 M KCl agar bridge.

**Perforated Patch Technique**

Volume measurements initially were attempted during ruptured-patch voltage clamp, but this approach proved unsuitable. As
previously described for both cardiac myocytes (Sorota, 1992) and other cell lines (Worrell et al., 1989; Doroshenko and Neher, 1992; Kinard and Satin, 1995), isosmotic pipette and bath solutions sometimes precipitated unpredictable cell swelling and changes in whole-cell currents. To prevent this problem, the amphotericin perforated patch technique (Horn and Marty, 1988; Rae et al., 1991) was employed. Both cell volume and whole-cell currents were stable under perforated patch recording conditions (Sorota, 1992; Hall et al., 1995).

Amphotericin-B (Sigma Chemical Co., St. Louis, MO) was freshly dissolved in dimethylsulfoxide, and then diluted in electrode filling solution to give final amphotericin and DMSO concentrations of 100 μg/ml and 0.2% (vol/vol), respectively. The electrode tip was dipped into amphotericin-free filling solution for 2 s, and then the barrel was backfilled with amphotericin-containing solution. Filled pipettes were quickly attached to a Ag/AgCl half-cell, placed in the bath solution, and zeroed. Gigascals were formed without application of negative pressure, typically 30–45 s after backfilling the pipette. To follow the formation of amphotericin pores, access resistance and cell capacitance were monitored with 10-nA hyperpolarizing pulses from Eh. After ~20 min, access resistance fell to 7–10 MΩ, and cell capacitance was 70–200 pF.

The cation/anion permeability ratio of a perforated patch depends on characteristics of both the ionophore and endogenous ion channels. Since the permeability ratio affects the transport numbers for ion fluxes between the pipette and cytoplasm, it may affect the cell volume attained under voltage clamp (see discussion). The PK/PCl ratio of amphotericin-treated cardiac sarclemma was evaluated by exposing the entire sarclemma to ionophore and determining the zero current potential under current clamp. For these measurements, the ruptured patch procedure was exploited. Seals were made while myocytes were incubated in a solution containing (mM): 140 KCl, 3 MgSO4, 5 HEPES, 10 glucose, pH 7.4; this represented the pipette filling solution in perforated with NMDG) or Cl

**Statistics**

Data are reported as mean ± SEM; n represents the number of cells. Except for Fig. 1, which depicts voltage clamp data from a typical experiment, all I-V relationships are averages, and mean current densities are expressed in pA/pF to account for differences in cell membrane area. When multiple comparisons were made, data were subjected to analysis of variance. Bonferroni’s method for group comparisons was performed when appropriate. For simple comparisons, the Student’s t test was used. All statistical analyses were conducted in SIGMASTAT (SPSS).

**RESULTS**

**Characterization of Stretch-activated Currents**

Membrane currents elicited by hyposmotic cell swelling initially were studied by applying step and ramp voltage-clamp protocols to the same cell. Results for a typical cell in standard bath solution are shown in Fig. 1. For the step protocol, Eh was −40 mV, and depolarizations to test potentials between −100 and +40 mV were applied under control conditions (1T, Fig. 1 A) and again 5 min after swelling in 0.6T hypotonic solution (Fig. 1 B). The currents obtained after osmotic stretch were much larger than those obtained in 1T solution. The effects are depicted more clearly in Fig. 1 C in which swelling-induced difference currents, measured as the current in 0.6T minus that in 1T, are plotted. The difference currents were time independent over most of the voltage range explored. Between 0 and +40 mV, however, osmotic stretch provoked an increasing outward current that approached steady state during the 500-ms pulse. This time-dependent current resembles the delayed rectifier, IK, which is augmented by osmotic and hydrostatic cell swelling of guinea pig myocytes (Sasaki et al., 1992, 1994; Rees et al., 1995; Wang et al., 1996).

After recovery in 1T solution, the same cell was studied using the ramp protocol (28 mV/s) to define the steady state I-V relationship. As before, the currents in 0.6T were larger than those in 1T (Fig. 1 E). The I-V relationship for the osmotic stretch-induced difference current (Fig. 1 F, solid line) exhibited strong inward rectification at negative potentials and weaker outward rectification at positive potentials. Fig. 1 F also compares the results of the two voltage-clamp protocols. Steady state difference currents measured using the step protocol (●, data from Fig. 1 C) are superimposed.
on the difference current obtained with the ramp protocol. The good agreement verifies the adequacy of ramp protocols for determining swelling-induced steady state currents. Ramp clamps were used in subsequent experiments that were designed to study maintained currents that are likely to contribute to cell volume regulation.

Previous work on mammalian cardiac myocytes has described both a Gd$^{3+}$-sensitive cation channel in cell-attached and excised patches that is activated by pipette suction (Bustamante et al., 1991) or mechanical stretch (Sadoshima et al., 1992) and a Gd$^{3+}$-sensitive osmotic swelling of intact, unclamped cells (Suleymanian et al., 1995). Fig. 2 shows that Gd$^{3+}$ also blocked a component of whole-cell current elicited by osmotic swelling and decreased cell volume under perforated patch conditions. $E_m$ was held at −40 mV except during voltage ramps. During the initial 20 min control period in 1T, the I-V relationship was stable (Fig. 2 A, curve a), and no change in cell volume occurred (Fig. 2 D). Exposure of myocytes to 0.6T solution for 5 min increased cell volume by 37 ± 2%, a swelling similar to that previously observed in intact, unclamped rabbit ventricular myocytes (e.g., 34 ± 2%; Drewnowska and Baumgarth, 1991). Both inward current at negative and outward current at positive potentials simultaneously increased in amplitude, at −100 mV from −1.68 ± 0.19 to −7.55 ± 0.21 pA/pF and at +40 mV from +0.74 ± 0.05 to +3.82 ± 0.14 pA/pF (Fig. 2 A). After recovery of cell volume and current in 1T solution, the osmotic challenge was repeated in the presence of 10 μM Gd$^{3+}$ (Fig. 2 B; 1T, curve c 0.6T, curve d). Osmotic swelling still affected membrane currents after Gd$^{3+}$ treatment, but the inward shift of the I-V relationship at negative potentials in 0.6T solution was less pronounced (e.g., from −1.63 ± 0.19 to −3.54 ± 0.18 pA/pF at −100 mV; Fig. 2 B). Furthermore, Gd$^{3+}$ decreased the amount of cell swelling significantly; relative cell volume in 0.6T was 1.37 ± 0.02 in the absence of Gd$^{3+}$ and 1.33 ± 0.01 in its presence ($P < 0.05$). The Gd$^{3+}$-sensitive difference currents in 0.6T and 1T solution are plotted in Fig. 2 C. During osmotic stretch in 0.6T solution, Gd$^{3+}$ blocked (Fig. 2 C, curves b − d) an inwardly rectifying current that reversed near −57 mV. Inward rectification also is exhibited by a Gd$^{3+}$-sensitive cation SAC studied at the single channel level in chick heart (Ruknudin et al., 1993), but other SACs in chick and rat heart have a linear I-V relationship under nearly symmetrical and asymmetrical conditions (Craelius et al., 1988; Ruknudin et al., 1993). In contrast, Gd$^{3+}$ had no effect on currents in 1T (Fig 2 C, curves a − c). This argues that Gd$^{3+}$ blocked only a swelling-induced component of steady state current. Gd$^{3+}$ did not block all of the swelling-induced current, however. The outwardly rectifying component at positive potentials was unaffected (compare Fig. 2, A and B). The Gd$^{3+}$-resistant current is likely to be the osmotic swelling-induced anion current, $I_{Gd swell}$ previously described in atrial, ventricular, and SA nodal myocytes from dog (Tseng, 1992; Sorota, 1992), rabbit (Hagiwara et al., 1992; Duan et al., 1995), rat (Coulombe and Coraboeuf, 1992), guinea pig (Vandenberg et al., 1994; Shuba et al., 1996), and man (Oz and Sorota, 1995; Sakai et al., 1995).

Although intracellular K$^+$ is controlled by dialysis across the perforated patch, osmotic swelling initially dilutes intracellular K$^+$ and the resulting shift in $I_{K1}$ might be mistaken for a novel swelling-activated cation current. To test whether $I_{K1}$ contributes to the Gd$^{3+}$-sensitive current, Ba$^{2+}$, a potent blocker of $I_{K1}$ in rabbit ventricular myocytes (Giles and Imaizumi, 1988; Shi-

![Figure 1](image_url). Currents activated on cell swelling. Voltage steps were applied to characterize whole-cell currents under isotonic (A, 1T) and hypotonic (B, 0.6T) conditions. (C) Difference currents ($I_{0.6T} - I_{1T}$) for step protocol shown in D. Swelling in 0.6T solution increased the currents at the end of 500-ms pulses. (E) Same cell studied in 1T and 0.6T solutions with a ramp protocol (28 mV/s). Hypotonic solution induced an inwardly rectifying current at negative potentials and an outwardly rectifying current at positive potentials. (F) Difference current elicited with voltage ramps (solid line). Steady state step difference currents (■, from C) superimpose on ramp difference current. (G) Diagram of ramp protocol.
moni et al., 1992), was employed. Under isosmotic conditions, 0.2 mM Ba\(^{2+}\) markedly attenuated the inward rectification of the whole-cell current at negative potentials attributable to \(I_{K1}\) (Fig. 3 A).\(^2\) Nevertheless, swelling in 0.6T + Ba\(^{2+}\) still turned on an inwardly rectifying current (Fig. 3 B, curve c) that was blocked by 10 \(\mu M\) Gd\(^{3+}\) (Fig. 3 B, curve d). The magnitude of the swelling-activated, Gd\(^{3+}\)-sensitive current in the presence of Ba\(^{2+}\), \(-4.29 \pm 0.30\) pA/pF at \(-100\) mV (Fig. 3 C, curve c – d) was similar to that in the absence of Ba\(^{2+}\), \(-4.01 \pm 0.23\) pA/pF (Fig. 2 C, curve b – d). These data argue that \(I_{K1}\) does not significantly contribute to the swelling-activated Gd\(^{3+}\)-sensitive current.

**Kinetics of Current Activation**

The kinetics of current activation also suggested that separate processes contribute to osmotic swelling-induced currents and positive and negative potentials. To examine activation kinetics, I-V relationships were determined every 30 s during swelling in 0.6T and recovery in 1T solutions in the absence and presence of Gd\(^{3+}\). The currents at \(+40\) and \(-100\) mV are plotted in Fig. 4 A. As expected from Fig. 2, the current at \(+40\) mV was resistant to Gd\(^{3+}\), whereas the current at \(-100\) mV consisted of nearly equal Gd\(^{3+}\)-sensitive and -insensitive components. The kinetics of current activation are shown more clearly in Fig. 4, B and C, in which the amplitude of swelling-activated current is expressed as a percentage of the maximum swelling-activated current. Upon exposure to 0.6T solution, the inwardly rectifying current at \(-100\) mV activated significantly more rapidly than the outwardly rectifying current at \(+40\) mV (Fig. 4 B); the \(t_{1/2}\)s were 18.7 \pm 1.8 and 55.2 \pm 3.4 s, respectively \((P < 0.05)\). This difference in \(t_{1/2}\) was eliminated when the 0.6T osmotic challenge was repeated.

---

\(^2\)\(I_{K1}\) current density in 1T, estimated as Ba\(^{2+}\)-sensitive current, was less than that recorded by Giles and Imaizumi (1988) and Shimoni et al. (1992) in rabbit ventricular cells. This difference may arise from the methods. They used ruptured rather than perforated patch. Concentrations of inorganic and organic modulators of \(I_{K1}\), including Mg\(^{2+}\) and polyamines (Nichols and Lopatin, 1997), are likely to differ. Furthermore, greater cell capacitance, cell diameter, and animal weight suggest the present experiments used older animals or selected a different population of ventricular cells.
in the presence of 10 μM Gd³⁺. Gd³⁺ slowed the t₁/₂ for
current activation at −100 mV to 46.3 ± 3.1 s without
significantly affecting activation at +40 mV, 53.8 ± 3.5 s;
as a result, the t₁/₂ for inward and outward current ac-
tivation no longer were significantly different (P > 0.21).
Thus, Gd³⁺ preferentially affected an inwardly rectifying
component of osmotic stretch-induced current that
activated more rapidly than the rest of the current. Sim-
ilar results were obtained when the kinetics of current
inactivation upon returning the cells to isotonic (1T)
solution were considered. Half-times of activation and
inactivation are summarized in Table I. Slow activation
of swelling-induced outward current was noted previ-
ously (Sorota, 1995).

**Ionic Nature of Gd³⁺-sensitive Current**

Whereas Gd³⁺ generally is held to be a blocker of cat-
ion SACs (Yang and Sachs, 1989; Hamill and McBride,
1996), Robson and Hunter (1994) describe a direct ef-
effect of Gd³⁺ on swelling-activated Cl⁻ currents in Rana
temporaria proximal tubule cells, and Zhang et al.
(1994) and Zhang and Lieberman (1996) describe a
Ca²⁺-dependent indirect effect on swelling-activated
Cl⁻ currents in chick myocytes. The experiments de-
picted in Fig. 4 were conducted in Ca²⁺-free bathing
media. Nevertheless, the same Gd³⁺-sensitive and -resis-
tant current components were observed as in the pres-
ence of bath Ca²⁺ (see Fig. 2). This argues that swell-
ing-activated currents in mammalian myocytes are not
Ca²⁺ dependent (Tseng, 1992; Sorota, 1992; Hagiwara
et al., 1992), and we previously showed that the amount
of swelling in hypotonic solution is not Ca²⁺ dependent
(Suleymanian et al., 1995).

To exclude the possibility that the Gd³⁺-sensitive cur-
rent was carried by Cl⁻, cells were studied using Cl⁻-
free (methanesulfonate) bath and low Cl⁻ (aspartate;
Cl⁻ = 5 mM) pipette solutions. The I-V relationships
depicted in Fig. 5 A were obtained in Cl⁻-free 1T (Fig.
5 A, curve a) and 0.6T (Fig. 5 A, curve b) solutions with-
out Gd³⁺. Osmotic swelling still increased the current
amplitude at negative potentials, but removal of Cl⁻
sharply attenuated the outwardly rectifying current at
positive potentials (see Fig. 2 A). The remaining swell-
ing-activated current was blocked almost completely by
10 μM Gd³⁺ (Fig. 5 B; Cl⁻-free 1T, curve c; Cl⁻-free
0.6T, curve d). On the other hand, removal of Cl⁻ had
no effect on the Gd³⁺-sensitive, inwardly rectifying dif-
cence current in 0.6T (Fig. 5 C, curve b – d), or on the
reduction of cell swelling caused by adding Gd³⁺ to
0.6T solution (Fig. 5 D). As before, Gd³⁺ was efficacious
only after cell swelling; Gd³⁺ failed to alter either cell
volume or the I-V relationship in Cl⁻-free 1T solution,
and the Gd³⁺-sensitive difference current was negligi-
bile (Fig. 5 C, curve a – c). These data suggest that Cl⁻
contributed to the outwardly rectifying current evoked
by hypotonic solution, but was not a significant compo-
nent of the Gd³⁺-sensitive, inwardly rectifying current.

If physiologic cations are the major charge carriers of
the Gd³⁺-sensitive, osmotic stretch-activated inward rec-
tifier, removal of Na⁺, K⁺, and Ca²⁺ from the bath and
electrode solutions should markedly attenuate both the
inwardly rectifying current in hypotonic solution and
the Gd\(^{3+}\) sensitivity of the remaining current. These predictions were tested by replacing Na\(^+\) and K\(^+\) in the bath with NMDG and in the pipette with Cs\(^+\) and replacing bath Ca\(^{2+}\) with Mg\(^{2+}\). Fig. 6 A depicts the I-V relationships in 1T, 0.6T, and 0.6T plus Gd\(^{3+}\) (Fig. 6 A, curves a–c, respectively) NMDG solutions. Hypotonic NMDG solution still provoked outwardly rectifying current at positive potentials. As predicted, however, the inward current was abolished (see Figs. 1 and 5 A); instead, the inward current was linear. Moreover, 10 \(\mu\)M Gd\(^{3+}\) did not significantly alter the I-V relationship in NMDG-0.6T solution; the negligible Gd\(^{3+}\)-sensitive difference current is shown in Fig. 6 B, curve b–c. Cation replacement also affected the cell volume response. Gd\(^{3+}\) no longer reduced cell volume in 0.6T NMDG solution (Fig. 6 C; compare Figs. 2 D and 5 D). These data strongly argue that the inwardly rectifying current induced by osmotic swelling was carried by cations. Therefore, the Gd\(^{3+}\)-sensitive current was designated \(I_{\text{Cl,swell}}\) (cation inward rectifier, swelling activated).

The suggestion that the Gd\(^{3+}\)-resistant current elicited by 0.6T solution is \(I_{\text{Cl,swell}}\) also was supported by experiments in NMDG solutions. Under these conditions, the \(I_{\text{Cl,swell}}\) blocker 9-AC (1 mM) did not affect the I-V relationship in 1T solution when SACs should be closed (data not shown). On the other hand, 9-AC totally prevented activation of the remaining swelling-induced current in 0.6T NMDG solution (Fig. 6 A, curve d; compare 1T, curve a). The 9-AC-sensitive current (Fig. 6 B, curve e–d) exhibited outward rectification at positive potentials and a reversal potential (\(E_{\text{rev}}\)) of \(-35.6 \pm 1.8 \text{ mV}\). Assuming equilibration across the perforated patch, \(E_{\text{Cl}}\) calculated from the bath and pipette solutions was \(-32.9 \text{ mV}\) after accounting for the effect of ionic strength on activity coefficients (\(\gamma_{\text{Cl,bath}} = 0.78\); \(\gamma_{\text{Cl,cell}} = 0.74\)). The small difference between \(E_{\text{rev}}\) and the calculated \(E_{\text{Cl}}\) is likely to be due in part to imperfect selectivity of stretch-activated anion channels (Tseng, 1992; Sorota, 1992; Hagiwara et al., 1992; Zhang et al., 1993; Vandenberg et al., 1994) and difficulty in controlling intracellular Cl\(^{-}\) with the perforated patch method. Furthermore, Fig. 6 C shows that 9-AC signifi-

![Figure 5](image-url)  
**Figure 5.** Replacement of bath Cl\(^{-}\) with methanesulfonate did not affect Gd\(^{3+}\)-sensitive current. Average I-V relationships in Cl\(^{-}\)-free 1T (a and c) and Cl\(^{-}\)-free 0.6T (b and d) solutions in the absence (A) and presence (B) of 10 \(\mu\)M Gd\(^{3+}\); \(n = 5\) cells. Hypotonicity elicited an inwardly rectifying current (A, b) that was inhibited by Gd\(^{3+}\) (B, d). (C) Gd\(^{3+}\)-sensitive currents under isotonic (a–c) and hypotonic (b–d) conditions. (D) After replacing Cl\(^{-}\), Gd\(^{3+}\) still reduced cell swelling in 0.6T solution. \(E_{\text{rev}}\) = -40 mV, bath, Cl\(^{-}\)-free, Ca\(^{2+}\)-free; pipette, low Cl\(^{-}\).

![Figure 6](image-url)  
**Figure 6.** Gd\(^{3+}\)-sensitive current is cation-dependent. Na\(^+\) and K\(^+\) were replaced with N-methyl-glucamine and Ca\(^{2+}\) with Mg\(^{2+}\). (A) Average I-V relationships in 1T (a) and 0.6T in the absence (b) and presence (c) of 10 \(\mu\)M Gd\(^{3+}\), and in 0.6T with Gd\(^{3+}\) plus 1 mM 9-AC (d); \(n = 5\) cells. (B) Gd\(^{3+}\)-sensitive current (b–c) was negligible in 0.6T NMDG solution. In contrast, 9-AC blocked an outwardly rectifying current (e–d). (C) Gd\(^{3+}\) did not affect cell volume in 0.6T NMDG solution, but 9-AC increased swelling. I-V relationships shown in A were obtained at times a–d. \(E_{\text{rev}}\) = -40 mV, pipette, Na\(^+\) and K\(^+\) replaced with Cs\(^{+}\).
Table II. A 13-fold increase of $[K^+]_o$ of sulfonate. The difference currents (0.6T and K curve $d$) was raised from 5 to 35 and 65 mM by equimolar replacement of Na$^+$ (b – a); 35 K$^+$, 35 Na$^+$ (d – c); and 65 K$^+$, 5 Na$^+$ (f – e); n = 5 cells. Although inward rectification was more pronounced, the magnitude of the inward current at diastolic potentials was smaller in 5 mM K$^+$ than in 35 and 65 mM K$^+$. (B) Concurrent recordings of cell volume. Increasing bath K$^+$ resulted in greater swelling in 0.6T solution. I-V relationships were measured at times indicated by a – f. (C) Relationship between swelling-induced current at $-80$ mV and relative cell volume in 0.6T was linear. Summary data for $E_{rev}$, $P_K/P_{Na}$ ratio, conductance at $E_{rev}$, and rectifier ratio are presented in Table II. Data from Fig. 7. $P_K/P_{Na}$ ratio was calculated from $E_{rev}$ and ionic concentrations, $g_{Cir,swell}$ from $I_{Cir,swell}$ at $E_{rev} = 10$ mV, and rectifier ratio as absolute value of ratio of $I_{Cir,swell}$ at $E_{rev} = 40$ mV; n = 5.

| $[K^+]_o$ | $[Na^+]_o$ | $E_{rev}$ | $P_K/P_{Na}$ | $g_{Cir,swell}$ | Rectifier ratio |
|-----------|------------|-----------|---------------|-----------------|----------------|
| 5         | 65         | $-56.1 \pm 2.3$ | 6.3 $\pm 0.5$ | 31 $\pm 2$ & 3.0 $\pm 0.2$ |
| 35        | 35         | $-31.3 \pm 1.9$ | 6.1 $\pm 0.4$ | 38 $\pm 3$ & 2.0 $\pm 0.2$ |
| 65        | 5          | $-19.2 \pm 1.3$ | 5.4 $\pm 0.6$ | 46 $\pm 3$ & 1.6 $\pm 0.3$ |

Data from Fig. 7. $P_K/P_{Na}$ ratio was calculated from $E_{rev}$ and ionic concentrations, $g_{Cir,swell}$ from $I_{Cir,swell}$ at $E_{rev} = 10$ mV, and rectifier ratio as absolute value of ratio of $I_{Cir,swell}$ at $E_{rev} = 40$ mV; n = 5.

Constant field $P_K/P_{Na}$ ratios calculated from $E_{rev}$ and ion concentrations and the rectifier ratio. For the three combinations of K$^+$ and Na$^+$, the $P_K/P_{Na}$ ratio was 5.9 ± 0.3, indicating only a modest selectivity for K$^+$, and increasing K$^+$ decreased the extent of rectification by approximately twofold. Because extracellular Na$^+$ is much greater than K$^+$ under physiologic conditions, inward current normally would be carried predominantly by Na$^+$, $P_K/P_{Na}$ ratios of 0.7–7.2 have been estimated for Gd$^{3+}$-sensitive unitary cation SAC currents elicited in chick ventricle by pipette suction (Bustamante et al., 1991; Ruknudin et al., 1993). The selectivity of the present channel for Ca$^{2+}$ was not examined, although Ca$^{2+}$ is known to permeate cation SACs in a number of tissues, including the heart (Sigurdson et al., 1992).

Replacing Na$^+$ with K$^+$ not only altered the difference current, it also caused cell swelling in 0.6T solution to significantly increase from 1.279 ± 0.021 to 1.345 ± 0.018, and 1.428 ± 0.020 in 5, 35, and 65 mM [K$^+]_o$ bathing media, respectively. The relationship between the current induced by swelling and cell volume in 0.6T solution is illustrated in Fig. 7 C. The data were well described by a straight line with a slope of 0.048 and a y-intercept of 1.22 ($r = 0.99$).

**Dose Dependence of Gd$^{3+}$**

Yang and Sachs (1989) reported that 10 μM Gd$^{3+}$ fully blocks stretch-activated cation channels in frog oocytes, but Bustamante et al. (1991) indicated a 10-fold higher dose is necessary in chick and guinea pig myocytes. To characterize the dose dependence of Gd$^{3+}$’s effects on current and volume, cells swollen in Cl$^-$-free 0.6T bath solution were treated with successively higher concentrations of Gd$^{3+}$ (1–30 μM). Fig. 8 A shows $I_{Cir,swell}$ (curve b – a) and the effect of 30 μM Gd$^{3+}$ (curve f – a). This dose of Gd$^{3+}$ appears to have totally blocked the inward rectifier, leaving a small, nearly linear, swelling-induced current. The residual current is likely to reflect in part the permeation of anions other than Cl$^-$ through the swelling-activated anion channel. The Gd$^{3+}$-sensitive current in 0.6T solution is depicted in Fig. 8 B. As the Gd$^{3+}$ concentration was increased, the amount of current blocked by Gd$^{3+}$ increased significantly ($P < 0.01$).
The effect of Gd³⁺ on cell volume in 0.6T solution also was dose dependent (Fig. 8 C). As the Gd³⁺ concentration was increased, relative cell volume in 0.6T solution significantly decreased ($P < 0.001$).

Dose-response curves for current blocked by Gd³⁺ at −80 mV and the reduction of cell volume caused by Gd³⁺ in 0.6T solution are presented in Fig. 9 A. The data from Fig. 8, B and C were fitted to the Hill equation, and the responses are plotted with the fitted maximum taken as 100%. The $K_{0.5}$ and Hill coefficient, $n$, were: 1.7 ± 0.3 and 1.7 ± 0.4 μM ($r = 0.95$) for block of current (C) and 1.8 ± 0.4 and 1.3 ± 0.2 μM ($r = 0.96$) for the Gd³⁺-induced cell shrinkage (A). This means that the 10-μM dose of Gd³⁺ used in other experiments should have blocked 97% of the Gd³⁺-sensitive current and caused 92% of the maximum volume change.

The excellent correlation between the effects of Gd³⁺ on current and volume are emphasized in Fig. 9 B, in which responses were expressed as a percentage of the maximum. The relationship was well described by a straight line with a slope 0.87 and a y-intercept of 8.6%

For comparison of Gd³⁺-induced volume changes. Data from Fig. 8 replotted with response ($R$) expressed as a percentage of maximum response ($R_{\text{max}}$) at 30 μM Gd³⁺). (A) Fit of dose-response relationships for block of current (C) and reduction of volume (A) to Hill equation: $R_{\text{max}} (R/R_{\text{max}}) = C_{s} (K_{s}^* + C^*)^n$, where $C_{s}$ is the Gd³⁺ concentration, $n$ is the Hill coefficient, $K^*$ is the apparent constant, and $K_{0.5}$ equals ($K^* / n$). $K_{0.5}$ and $n$ for current and cell volume were 1.7 ± 0.3 μM and 1.7 ± 0.4, and 1.8 ± 0.4 μM and 1.3 ± 0.2, respectively. (B) Relationship between block of current and reduction of cell volume was linear. ($r = 0.98$). The slope and y-intercept were not significantly different than 1 and 0, respectively.

**Graded Activation of Gd³⁺ Sensitivity**

Gd³⁺-sensitive cation SAC activity in cell-attached patches is a graded function of the negative pressure applied to the pipette and the degree of membrane stretch (Guharay and Sachs, 1984; Sigurdson et al., 1987). For a range of stimuli, the open probability of the nonselective cation mechanoelectrical transduction channel in the bullfrog saccular hair cell has been postulated to be linearly related to membrane tension (Howard et al., 1988), whereas Guharay and Sachs (1984) postulated that gating of the cation SAC in tissue-cultured embryonic chick skeletal muscle cells varies with the square of membrane tension. One might expect the magnitude of $I_{\text{Gd,swell}}$ and Gd³⁺-induced volume changes also would depend on the amount of swelling-induced membrane stretch. To test this idea, myocytes were placed in a series of hypotonic (0.6–0.9T), isotonic (1T), and hypertonic (1.5T) solutions, and the I-V relationship and relative cell volume were monitored. $I_{\text{Gd,swell}}$ was measured as the difference between the I-V relationships ± 10 μM Gd³⁺ in each of the superfusates. Fig. 10 A shows that Gd³⁺ did not affect membrane current in isotonic solution or when myocytes were shrunken in 1.5T solution. On the other hand, as bath solution osmolarity was gradually stepped from 1 to 0.6T, $I_{\text{Gd,swell}}$ increased in a graded fashion. At the same time, osmotic swelling in the presence of Gd³⁺ was attenuated (Fig. 10 B). These effects are summarized in Fig. 10 C where Gd³⁺-sensitive current and cell shrinkage are plotted versus bath osmolarity.

Because activation of $I_{\text{Gd,swell}}$ should depend on cell volume rather than osmolarity per se, Gd³⁺-sensitive
current at −80 mV and Gd³⁺-induced cell shrinkage are plotted as a function of relative cell volume before adding Gd³⁺ in Fig. 11, A and B. Both effects of Gd³⁺ were apparent in 0.9T solution at a relative volume of 1.075 ± 0.025, the smallest amount of swelling examined, and were fully activated in 0.7T solution at a relative volume of 1.305 ± 0.033. Decreasing bath osmolarity to 0.6T did not alter the responses to Gd³⁺ despite causing additional cell swelling. Furthermore, the Gd³⁺-sensitive current and shrinkage, expressed as a percentage of the maximum Gd³⁺-induced change, were proportional over a wide range of osmolarities (0.6–1.5T). This linear relationship (Fig. 11 C) suggests a tight coupling between the processes.

Kinetics of Gd³⁺’s Effects on Current and Volume

The data in Figs. 9 B and 11 C establish that block of current by Gd³⁺ and cell shrinkage are linearly related. It is unclear, though, whether block of current causes cell shrinkage or whether the primary effect of Gd³⁺ is cell shrinkage, which in turn is responsible for the diminution of current. This question can be addressed by examining the kinetics of the two effects. Fig. 12 depicts whole-cell current at −80 mV (○) and cell volume (▲) at 20-s intervals during osmotic swelling and exposure to 10 μM Gd³⁺. Gd³⁺ decreased the inward current with a t₁/₂ of 50.3 ± 2.5 s (Fig. 12, down arrow), whereas the Gd³⁺-induced cell shrinkage was significantly slower, with a t₁/₂ of 116.3 ± 3.4 s (up arrow). These data argue that the primary effect of Gd³⁺ is block of I_{Gr,swell} rather than cell shrinkage. They do not, however, rule out the possibility that cell shrinkage secondary to block of current leads to a further reduction of I_{Gr,swell}.

Voltage Dependence of the Sensitivity of Cell Volume to Gd³⁺

Gd³⁺-sensitive I_{Gr,swell} was larger at physiologically relevant diastolic potentials than at plateau voltages. At −80 mV, for example, the Gd³⁺-sensitive current was >2 pA/pF in 0.6T solution (Fig. 10 A). A sustained current of this magnitude represents a significant transmembrane ion flux and could cause significant alterations in intracellular osmolarity and, thus, cell volume. Because of its inward-going rectification, however, the Gd³⁺-sensitive current should have little effect on cell volume at more positive potentials. To investigate this idea, Eₒ was varied from −80 and −40 mV under isosmotic conditions (1T) and during hypotonic cell swelling (0.6T) in the absence and presence of 10 μM Gd³⁺.

Figure 10. Response to Gd³⁺ depends on bath osmolarity. I-V relationships and cell volumes were determined in the absence and presence of 10 μM Gd³⁺ in 0.6T–1.5T bath solutions, n = 5 cells. (A) Average Gd³⁺-sensitive currents. (B) Cell volumes in the absence and presence of Gd³⁺; cell volumes in hypotonic solution were reduced by Gd³⁺. Both the Gd³⁺-sensitive current and the Gd³⁺-induced reduction of volume progressively increased as bath osmolarity was decreased; maximum responses were observed in ≥0.7T. In contrast, Gd³⁺ did not alter the I-V relationship or cell volume in isotonic (1T) or hypertonic (1.5T) solutions. (C) Gd³⁺-sensitive current and cell shrinkage are plotted as a function of bath-relative osmolarity. Eₒ, −80 mV; bath, 0 Cl⁻; pipette, low Cl⁻.

Figure 11. Response to Gd³⁺ depends on cell volume. Data taken from Fig. 10. (A) Gd³⁺-sensitive current at −80 mV and (B) Gd³⁺-induced cell shrinkage are plotted as functions of relative cell volume before adding Gd³⁺ (volumes normalized to control, 1T). Maximum responses to Gd³⁺ were observed when relative cell volume was 1.305 ± 0.033 in 0.7T solution. (C) Gd³⁺ block of current and Gd³⁺-induced reduction of cell volume were linearly related.
Gd$^{3+}$. The I-V relationships for the Gd$^{3+}$-sensitive current holding at $-40$ mV (Fig. 13 A; 1T, curve $a$–$e$; 0.6T, curve $b$–$f$) and at $-80$ mV (Fig. 13 B; 1T, curve $d$–$k$; 0.6T, curve $c$–$g$) are plotted. As expected, the Gd$^{3+}$-sensitive current in 0.6T was not affected by $E_h$. Relative cell volume during the protocol is shown in Fig. 13 C. If Gd$^{3+}$ blocks an inwardly rectifying cation current, cell volume in 0.6T plus Gd$^{3+}$ should be less than in 0.6T without Gd$^{3+}$ at $-80$ mV (see Fig. 13 C, $c$ and $g$), but because depolarization limits the current, Gd$^{3+}$ should fail to alter cell volume at $-40$ mV (see Fig. 13 C, $b$ and $f$). Both of these predictions were observed. The Gd$^{3+}$-sensitive current was not the only mechanism regulating volume under these conditions, however. Hyperpolarization from $-40$ to $-80$ mV in 0.6T without Gd$^{3+}$ should lead to the activation of inward current and cell swelling at $-80$ mV if this current acted alone. Instead, a very small cell shrinkage was observed (see Fig. 13 C, $b$ and $c$). This suggests that the osmotic effect of Gd$^{3+}$-sensitive cation influx was opposed by a voltage-dependent efflux of osmolytes. When the cation influx was blocked by Gd$^{3+}$, a significant shrinkage was unmasked on hyperpolarization (see Fig. 13 C, $f$ and $g$). These portions of data can be explained by the contribution of $I_{\text{Cl,swell}}$ to osmolyte fluxes.

D I S C U S S I O N

Osmotic swelling of rabbit ventricular myocytes caused the graded activation of $I_{\text{Cl,swell}}$, a Gd$^{3+}$-sensitive cation current with a $P_K/P_Na$ ratio of ~6. $I_{\text{Cl,swell}}$ was not detectable under isosmotic conditions, but was readily measured after a swelling of only 7.5%, the smallest perturbation explored. The I-V relationship for $I_{\text{Cl,swell}}$ revealed a strong inward-going rectification and was insensitive to Ba$^{2+}$, a blocker of $I_K$. This swelling-induced current appeared to be time dependent at potentials negative to 0 mV. Several lines of evidence indicate that $I_{\text{Cl,swell}}$ modulates cell volume at physiologically relevant potentials. (a) A linear relationship was found between the amount of $I_{\text{Cl,swell}}$ blocked by Gd$^{3+}$ during graded osmotic swelling and with varying concentrations of Gd$^{3+}$ and the subsequent Gd$^{3+}$-induced reduction of cell volume. In 0.6T, 10 μM Gd$^{3+}$ reduced swelling by ~30%, and Gd$^{3+}$-induced block of $I_{\text{Cl,swell}}$ preceded Gd$^{3+}$-induced cell shrinkage by ~1 min. (b) Elimination of the Gd$^{3+}$-sensitive current in hypotonic solution containing NMDG instead of Na$^+$ and K$^+$ also eliminated the effect of Gd$^{3+}$ on cell volume. (c) The amount of swelling in hypotonic solution was linearly related to $I_{\text{Cl,swell}}$ when bath Na$^+$ was partially replaced by K$^+$. (d) Gd$^{3+}$ also reduces cell volume in osmotically swollen, unclamped myocytes (Suleymanian et al., 1995). These data indicate that osmotic swelling elicited a cation influx via a poorly selective channel. Rather than provoking an RVD, the influx of cations contributed to cell swelling.

A number of studies in mammalian myocytes focused on a swelling-induced, outwardly rectifying anion current, $I_{\text{Cl,swell}}$ (Tseng, 1992; Sorota, 1992, 1995; Hagiwara et al., 1992; Coulombe and Coraboeuf, 1992; Vandenberg et al., 1994; Duan et al., 1995; Shuba et al., 1996), and the characteristics of the 9-AC–sensitive $I_{\text{Cl,swell}}$ observed here were consistent with these reports. However, in-
sensitivity to Gd$^{3+}$ and to omission of extracellular Ca$^{2+}$
distinguishes I$_{Cl,swell}$ in rabbit from that found in chick
heart (Zhang et al., 1994). Although previous reports
suggested that brief pharmacological blockade of I$_{Cl,swell}$
does not affect cell volume (Tseng, 1992; Sorota,
1992), the present paradigm detected a 28% augmenta-
tion of cell swelling in hypotonic solution on block-
ing I$_{Cl,swell}$ with 9-AC, and the effect of 9-AC on cell vol-
ume under voltage clamp was similar to that observed
in unclamped myocytes (Suleymanian et al., 1995). A
9-AC-induced swelling was expected because inward
current carried by I$_{Cl,swell}$ at diastolic potentials repre-
sents the efflux of anions. It is uncertain why we ob-
erved increased swelling with 9-AC, whereas others did
not. Species differences cannot be ruled out. For exam-
ple, 9-AC (1 mM) blocks only 50–60% of I$_{Cl,swell}$ in ca-
nine and guinea pig myocytes (Tseng, 1992; Sorota,
1994; Vandenberg et al., 1994), but it blocked all of
I$_{Cl,swell}$ in rabbit ventricular (Fig. 6 A, a and d) and atrial
(Hagiwara et al., 1992) myocytes. In addition, the use
of ruptured patch technique (Tseng, 1992; Sorota, 1992)
may have blunted changes in intracellular osmolarity
caused by 9-AC. Finally, the present digital video micro-
scopy technique for estimating volume has a much
greater resolution than measurements of cell width with
an ocular reticle (Tseng, 1992; Sorota, 1992).

Basis for the Gd$^{3+}$-sensitive Swelling-activated Cation Current

This appears to be the first description at the whole-cell
level of a Gd$^{3+}$-sensitive, poorly selective cation current
elicted by swelling cardiac myocytes. Single channel re-
cordings from chick, guinea pig, and rat cardiac cells
demonstrate that several different channel channels acti-
vated by pipette suction are blocked by Gd$^{3+}$ (Busta-
mante et al., 1991; Sadoshima et al., 1992; Ruknudin et
al., 1993). A 25-pS channel in chick heart exhibits
strong inward-going rectification that is unaffected by
switching Na$^+$ and K$^+$ on one side (Ruknudin et al.,
1993). In contrast, the rectification found here was
markedly diminished when bath Na$^+$ was replaced by
K$^+$ (Fig. 7 A). Other Gd$^{3+}$-sensitive cation and K$^+$
channels in chick (Ruknudin et al., 1993) and rat (Sa-
doshima et al., 1992) have linear unitary I-V relation-
ships in both symmetrical K$^+$ solutions and with Na$^+$ re-
placing K$^+$ on one side, and the voltage dependence of
their open probability would not generate inward recti-
fication of whole-cell currents. Thus, none of the Gd$^{3+}$-
sensitive SACs described at the single channel level can
fully account for the behavior of I$_{Cl,swell}$. Nevertheless,
the apparent $K_{0.5}$ for block of current and for Gd$^{3+}$-
induced cell shrinkage, 1.7 and 1.8 μM, and Hill coeffi-
cients of 1.7 and 1.3, were consistent with Gd$^{3+}$ block of
SACs in *Xenopus* oocytes (Yang and Sachs, 1989). Yang
and Sachs (1989) suggested that low concentrations of
Gd$^{3+}$ screen negative charges near the vestibule of the
channel and interact with an allosteric site outside the
membrane field to induce a short-lived closed state,
whereas higher concentrations cause a cooperative
transition to a long-lived closed state.

At positive potentials, an increasing component of
outward current also was observed during swelling (Fig.
1 C) and attributed to I$_{Cl,swell}$ because of its sensitivity
to 9-AC and Cl$^-$ replacement. Swelling-activated outward
current may reflect in part a stimulation of the delayed
rectifier, I$_K$, that previously was noted during both os-
monic and hydrostatic swelling of guinea pig ventricular
myocytes (Sasaki et al., 1992; 1994; Rees et al., 1995;
Wang et al., 1996). Both groups agree that swelling pri-
marily increases the slowly activating component, I$_{Ks}$,
but the rapidly activating component, I$_{Kr}$, is said to ei-
ther decrease (Rees et al., 1995) or increase (Wang et
al., 1996). It is possible that swelling-activated delayed
rectifier contributes to the total current in 0.6T, al-
though this current is much smaller in rabbit ventricle
than in several other species (Giles and Imaiuzumi,
1988). The time-dependent current observed here was
found at potentials more appropriate for I$_{Ks}$, although
its approaching steady state within 500 ms was suggest-
tive of I$_{Ko}$. To the extent I$_K$ contributes to swelling-
induced current, it also might contribute to the Gd$^{3+}$-
sensitive current. I$_{Ko}$ is blocked by ≥1 μM of another
lanthanide, La$^{3+}$, and ≥10 μM La$^{3+}$ shifts I$_{Ko}$ activa-
tion in a positive direction (Sanguinetti and Jurkiewicz,
1990). On the other hand, no component of current at-
tributable to block of I$_{Kr}$ or I$_{Ks}$ by Gd$^{3+}$ was present at
appropriate voltages.

Cell swelling or mechanical stretch also has been re-
ported to activate Gd$^{3+}$-insensitive, poorly selective, cat-
ion channels in neonatal rat atrial cells (Kim, 1993;
Kim and Fu, 1993). Such channels did not appear to be
present in adult rabbit ventricular cells. As judged by
the effect of replacement of Na$^+$ and K$^+$ with NMDG
and Ca$^{2+}$ with Mg$^{2+}$ in Cl$^-$free solution (Fig. 6, A and
B), all of the swelling activated cation current was
blocked by Gd$^{3+}$. Furthermore, the strong inward recti-
fication of I$_{Gr,swell}$ argues against the participation of
free fatty acid–activated (Kim, 1992) or ATP-sensitive
(Van Wagoner, 1993) K$^+$ channels in the response to
cell swelling. Activation of either of these channels
should have elicited a significant outward cation cur-
rent in 5 mM [K$^+$], but virtually none was observed.

Osmotic swelling may or may not be equivalent to
mechanical stretch or localized membrane deforma-
tion as a stimulus for activating SACs (Vandenberg et
al., 1996). Although both osmotic swelling and me-
chanical stimuli distort the membrane and cytoskele-
ton, osmotic swelling also dilutes intracellular ions and
macromolecules. Dilution of the intracellular contents
can modulate ion transport by multiple mechanisms.
change will occur. The other extreme assumes the pipette and membrane have opposite cation/anion selectivity. In such a case, total fluxes into or out of the cell are exactly twice the transmembrane flux, and observed volume changes are exactly twice those expected from transmembrane currents. The optimal situation is for the pipette cation and anion transport numbers both to equal 0.5. In that case, cation and anion fluxes between the pipette and cell are equal in magnitude but opposite in direction, and their effects on cell volume cancel. As a result, the observed volume change will exactly equal the change expected from the measured ionic current.

Unfortunately, the transport numbers of a perforated patch are difficult to predict. The fluxes are determined by the combined characteristics of the pore former and native membrane channels. Amphotericin pores are permeant to both monovalent cations and anions (Marty and Finkelstein, 1975; Horn and Marty, 1988; Ebihara et al., 1995; Kyrozis and Reichling, 1995), and amphotericin may affect the properties of native channels (Hsu and Burnette, 1993). In preliminary studies, $P_{K}/P_{Cl}$ of amphotericin-treated myocytes was estimated as 0.8–0.9 (see METHODS). Using $P_{K}/P_{Cl}$ to approximate $P_{cation}/P_{anion}$ and taking the potential across the perforated patch as $\sim 0$ mV, $t^+$ was $\sim 0.44–0.47$. Although only an approximation, these data suggest that the cation and anion fluxes between pipette and cell were roughly comparable and, therefore, that cell volume changes measured under perforated patch conditions should approximate those expected from the measured ionic currents.

**Physiological and Pathophysiological Implications**

Activation of $I_{Gr,swell}$ and $I_{Cl,swell}$ may directly affect cardiac electrical activity, alter ionic gradients, and con-
Swelling-activated Current and Myocyte Volume

This work was supported by National Heart, Lung, and Blood Institute grants HL-46764 and HL-02798.

Original version received 3 April 1997 and accepted version received 20 June 1997.

REFERENCES

Baumgarten, C.M., and J.J. Feher. 1998. Osmosis and the regulation of cell volume. In Cell Physiology Source Book, 2nd ed. N. Sperelakis, editor. Academic Press, Inc., San Diego, CA. 254–293.

Brezden, B.L., D.R. Gardner, and C.E. Morris. 1986. A potassium-selective channel in isolated Lymnaea stagnalis heart muscle cells. J. Exp. Biol. 125:175–189.

Bustamante, J.O., A. Ruknudin, and F. Sachs. 1991. Stretch-activated current and cell swelling in osmotically challenged adult rabbit ventricular myocytes. J. Gen. Physiol. 100:89–114.

Clemo, H.F., and C.M. Baumgarten. 1995. Gd\(^{3+}\) reduces a cation current and cell swelling in osmotically challenged adult rabbit ventricular myocytes. Biophys. J. 68:393a. (Abstr.)

Clemo, H.F., J.J. Feher, and C.M. Baumgarten. 1992. Modulation of rabbit ventricular cell volume and Na\(^+/K^+\)/2Cl\(^-\) cotransport by cGMP and atrial natriuretic factor. J. Gen. Physiol. 100:89–114.

Clemo, H.F., B.S. Stambler, and C.M. Baumgarten. 1995. Persistent stretch-activated current and cell volume in congestive heart failure. Circulation. 92:I–504. (Abstr.)

Drewnowska, K., H.F. Clemo, and C.M. Baumgarten. 1991. Prevention of myocardial intracellular edema induced by St. Thomas’ Hospital cardioplegic solution. J. Mol. Cell. Cardiol. 23:1215–1222.

Duan, D., B. Fermini, and S. Nattel. 1995. \(\alpha\)-Adrenergic control of volume-regulated Cl\(^-\) currents in rabbit atrial myocytes. Characterization of a novel ionic regulatory mechanism. Circ. Res. 77:379–393.

Ebihara, S., K. Shirato, N. Harata, and N. Akaike. 1995. Gramicidin-perforated patch recording: GABA response in mammalian neurons with intact intracellular chloride. J. Physiol. (Camb.) 484:77–86.

Golomb, E., and E. Coraboeuf. 1992. Large-conductance chloride channels of new-born rat cardiac myocytes are activated by hypotonic media. Pflügers Arch. 422:143–150.

Craeilius, W., V. Chen, and N. el-Sherif. 1988. Stretch activated ion channels in ventricular myocytes. Biochi. Rep. 8:407–414.

Doroshenko, P., and E. Neher. 1992. Volume-sensitive chloride conductance in bovine chromaffin cell membrane. J. Physiol. (Camb.) 449:197–218.

Drewnowska, K., and C.M. Baumgarten. 1991. Regulation of cellular volume in rabbit ventricular myocytes: bumetanide, chlorothiazide, and ouabain. Am. J. Physiol. 260:C122–C131.

Drewnowska, K., C.M. Baumgarten. 1991. Osmosis and the regulation of cell volume. In Cell Physiology Source Book, 2nd ed. N. Sperelakis, editor. Academic Press, Inc., San Diego, CA. 254–293.

Baumgarten, C.M., and J.J. Feher. 1998. Osmosis and the regulation of cell volume. In Cell Physiology Source Book, 2nd ed. N. Sperelakis, editor. Academic Press, Inc., San Diego, CA. 254–293.

Brezden, B.L., D.R. Gardner, and C.E. Morris. 1986. A potassium-selective channel in isolated Lymnaea stagnalis heart muscle cells. J. Exp. Biol. 125:175–189.

Bustamante, J.O., A. Ruknudin, and F. Sachs. 1991. Stretch-activated channels in heart cells: relevance to cardiac hypertrophy. J. Cardiovasc. Pharmacol. 17(Suppl. 2):110–113.

This work was supported by National Heart, Lung, and Blood Institute grants HL-46764 and HL-02798.

Original version received 3 April 1997 and accepted version received 20 June 1997.

REFERENCES

Baumgarten, C.M., and J.J. Feher. 1998. Osmosis and the regulation of cell volume. In Cell Physiology Source Book, 2nd ed. N. Sperelakis, editor. Academic Press, Inc., San Diego, CA. 254–293.

Brezden, B.L., D.R. Gardner, and C.E. Morris. 1986. A potassium-selective channel in isolated Lymnaea stagnalis heart muscle cells. J. Exp. Biol. 125:175–189.

Bustamante, J.O., A. Ruknudin, and F. Sachs. 1991. Stretch-activated channels in heart cells: relevance to cardiac hypertrophy. J. Cardiovasc. Pharmacol. 17(Suppl. 2):110–113.

Clemo, H.F., and C.M. Baumgarten. 1991. Atrial natriuretic factor decreases cell volume of rabbit atrial and ventricular myocytes. Am. J. Physiol. 260:C681–C690.

Clemo, H.F., and C.M. Baumgarten. 1995. Gd\(^{3+}\) reduces a cation current and cell swelling in osmotically challenged adult rabbit ventricular myocytes. Biophys. J. 68:393a. (Abstr.)

Clemo, H.F., J.J. Feher, and C.M. Baumgarten. 1992. Modulation of rabbit ventricular cell volume and Na\(^+/K^+\)/2Cl\(^-\) cotransport by cGMP and atrial natriuretic factor. J. Gen. Physiol. 100:89–114.

Clemo, H.F., B.S. Stambler, and C.M. Baumgarten. 1995. Persistent stretch-activated current and cell volume in congestive heart failure. Circulation. 92:I–504. (Abstr.)

Coulomb, E., and E. Coraboeuf. 1992. Large-conductance chloride channels of new-born rat cardiac myocytes are activated by hypotonic media. Pflügers Arch. 422:143–150.

Craeilius, W., V. Chen, and N. el-Sherif. 1988. Stretch activated ion channels in ventricular myocytes. Biochi. Rep. 8:407–414.

Doroshenko, P., and E. Neher. 1992. Volume-sensitive chloride conductance in bovine chromaffin cell membrane. J. Physiol. (Camb.) 449:197–218.

Drewnowska, K., H.F. Clemo, and C.M. Baumgarten. 1991. Prevention of myocardial intracellular edema induced by St. Thomas’ Hospital cardioplegic solution. J. Mol. Cell. Cardiol. 23:1215–1222.

Duan, D., B. Fermini, and S. Nattel. 1995. \(\alpha\)-Adrenergic control of volume-regulated Cl\(^-\) currents in rabbit atrial myocytes. Characterization of a novel ionic regulatory mechanism. Circ. Res. 77:379–393.

Ebihara, S., K. Shirato, N. Harata, and N. Akaike. 1995. Gramicidin-perforated patch recording: GABA response in mammalian neurons with intact intracellular chloride. J. Physiol. (Camb.) 484:77–86.
Fozzard, H.A., and C.O. Lee. 1976. Influence of changes in extracellular potassium and chloride ions on membrane potential and intracellular potassium ion activity in rabbit ventricular muscle. *J. Physiol. (Camb.)* 256:663–689.

Giles, W.R., and Y. Imaizumi. 1988. Comparison of potassium currents in rabbit atrial and ventricular cells. *J. Physiol. (Camb.)* 405:123–145.

Guharay, F., and F. Sachs. 1984. Stretch-activated single ion channel currents in tissue-cultured embryonic chick skeletal muscle. *J. Physiol. (Camb.)* 352:685–701.

Hagiwara, N., H. Masuda, M. Shoda, and H. Irisawa. 1992. Stretch-activated anion currents of rabbit cardiac myocytes. *J. Physiol. (Camb.)* 456:285–302.

Hall, S.K., J. Zhang, and M. Lieberman. 1995. Cyclic AMP prevents activation of a swelling-induced chloride-sensitive conductance in chick heart cells. *J. Physiol. (Camb.)* 488:359–369.

Hamill, O.P., and D.W. McBride. Jr. 1996. The pharmacology of mechanogated membrane ion channels. *Pharmacol. Rev.* 48:231–252.

Handy, J.R., Jr., B.H. Dorman, M.J. Cavallo, R.B. Hinton, R.C. Roy, F.A. Crawford, and F.G. Spinale. 1996. Direct effects of oxygenated crystalloid or blood cardioplegia on isolated myocyte contractile function. *J. Thorac. Cardiovasc. Surg.* 112:1064–1072.

Hansen, D.E., M. Borganelli, G.P. Stacy, Jr., and L.K. Taylor. 1991. Role of cell volume and protein kinase C in regulation of a Cl− conductance in single proximal tubule cells of Rana temporaria. *J. Physiol. (Camb.)* 480:1–7.

Ruknudin, A., F. Sachs, and J.O. Bustamante. 1993. Stretch-activated ion channels in tissue-cultured chick heart. *Am. J. Physiol.* 264:H960–H972.

Sachs, F. 1988. Mechanical transduction in biological systems. *Crit. Rev. Biomed. Eng.* 16:141–169.

Sackin, H. 1995. Mechanosensitive channels. *Annu. Rev. Physiol.* 57:333–353.

Sadoshima, J.-I., T. Takahashi, L. Jahn, and S. Izumo. 1992. Roles of mechanosensitive ion channels, cytoskeleton, and contractile activity in stretch-induced immediate-early gene expression and hypertrophy of cardiac myocytes. *Proc. Natl. Acad. Sci. USA.* 89:9905–9909.

Sakai, R., N. Hagiwara, H. Kasamuki, and S. Hosoda. 1995. Chloride conductance in human atrial cells. *J. Mol. Cell. Cardiol.* 27:2403–2408.

Sanguinetti, M.C., and N.K. Jurkiewicz. 1990. Lanthanum blocks a specific component of Iq and screens membrane surface charge in cardiac cells. *Am. J. Physiol.* 259:H1881–H1889.

Sasaki, N., T. Mitsuiye, A. Noma. 1992. Effects of mechanical stretch on membrane currents of single ventricular myocytes of guinea-pig heart. *Jpn. J. Physiol.* 42:957–970.

Sasaki, N., T. Mitsuiye, Z. Wang, and A. Noma. 1994. Increase of the delayed rectifier K+ and Na+-K+ pump currents by hypotonic solutions in guinea pig cardiac myocytes. *Circ. Res.* 75:887–895.

Schwiebert, E.M., J.W. Mills, and B.A. Stanton. 1994. Actin-based cytoskeleton regulates a chloride channel and cell volume in a renal cortical collecting duct cell line. *J. Biol. Chem.* 269:7081–7089.

Shimoni, Y., R.B. Clark, and W.R. Giles. 1992. Role of an inwardly rectifying potassium current in rabbit ventricular action potential. *J. Physiol. (Camb.)* 448:709–727.

Shubha, L.M., T. Ogura, and T.F. McDonald. 1996. Kinetic evidence distinguishing volume-sensitive chloride current from other types in guinea-pig ventricular myocytes. *J. Physiol. (Camb.)* 491:69–80.

Sigurdson, W.J., C.E. Morris, B.L. Brezden, and D.R. Gardner. 1987. Stretch activation of a K+ channel in molarcan heart cells. *J. Exp. Biol.* 127:191–209.

Sigurdson, W., A. Ruknudin, and F. Sachs. 1992. Calcium imaging of mechanically induced fluxes in tissue-cultured chick heart: role of stretch-activated ion channels. *Am. J. Physiol.* 262:H1110–H1115.

Sorota, S. 1992. Swelling-induced chloride-sensitive current in ca-
nine atrial cells revealed by whole-cell patch-clamp method. *Circ. Res.* 70:679–687.

Sorota, S. 1994. Pharmacologic properties of the swelling-induced chloride current of dog atrial myocytes. *J. Cardiovasc. Electrophysiol.* 5:1006–1016.

Sorota, S. 1995. Tyrosine protein kinase inhibitors prevent activation of cardiac swelling-induced chloride current. *Pflügers Arch.* 431:178–185.

Stacy, G.P., Jr., R.L. Jobe, L.K. Taylor, and D.E. Hansen. 1992. Stretch-induced depolarizations as a trigger of arrhythmias in isolated canine left ventricles. *Am. J. Physiol.* 263:H613–H621.

Sukharev, S.I., P. Blount, B. Martinac, H.R. Guy, and C. Kung. 1996. MscL: a mechanosensitive channel in *Escherichia coli*. *Soc. Gen. Physiol. Ser.* 51:133–141.

Suleymanian, M.A., and C.M. Baumgarten. 1996. Osmotic gradient-induced water permeation across the sarcolemma of rabbit ventricular myocytes. *J. Gen. Physiol.* 107:503–514.

Suleymanian, M.A., H.F. Clemo, N.M. Cohen, and C.M. Baumgarten. 1995. Stretch-activated channel blockers modulate cell volume in cardiac ventricular myocytes. *J. Mol. Cell. Cardiol.* 27:721–728.

Takano, H., and S.A. Glantz. 1995. Gadolinium attenuates the upward shift of the left ventricular diastolic pressure-volume relation during pacing-induced ischemia in dogs. *Circulation.* 91:1573–1587.

Tavi, P., M. Laine, and M. Weckström. 1996. Effect of gadolinium on stretch-induced changes in contraction and intracellularly recorded action- and after-potentials of rat isolated atrium. *Br. J. Pharmacol.* 118:407–413.

Tseng, G.N. 1992. Cell swelling increases membrane conductance of canine cardiac cells: evidence for a volume-sensitive Cl channel. *Am. J. Physiol.* 262:C1065–C1068.

Van Wagoner, D.R. 1993. Mechanosensitive gating of atrial ATP-sensitive potassium channels. *Circ. Res.* 72:973–983.

Wang, Z., T. Mitsuuye, and A. Noma. 1996. Cell distention-induced increase of the delayed rectifier K+ current in guinea pig ventricular myocytes. *Circ. Res.* 78:466–474.

Worrell, R.T., A.G. Butt, W.H. Cliff, and R.A. Frizzell. 1989. A volume-sensitive conductance in human colonic cell line T84. *Am. J. Physiol.* 256:C1111–C1119.

Yang, X.-C., and F. Sachs. 1989. Block of stretch-activated ion channels in *Xenopus* oocytes by gadolinium and calcium ions. *Science* (Wash. DC). 243:1068–1071.

Zhang, J., S.K. Hall, and M. Lieberman. 1994. An early transient current activates the swelling-induced chloride conductance in cardiac myocytes. *Biophys. J.* 66:A442. (Abstr.)

Zhang, J., and M. Lieberman. 1996. Chloride conductance is activated by membrane distention of cultured chick heart cells. *Cardiovasc. Res.* 32:168–179.

Zhang, J., R.L. Rasmusson, S.K. Hall, and M. Lieberman. 1993. A chloride current associated with swelling of cultured chick heart cells. *J. Physiol.* (Camb.). 472:801–820.