On the Mechanism of Rectification of the Isoproterenol-Activated Chloride Current in Guinea-Pig Ventricular Myocytes

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ABSTRACT The whole cell configuration of the patch clamp technique was used to investigate the mechanism underlying rectification of the isoproterenol-activated chloride (Cl^-) current in isolated guinea pig ventricular myocytes. When extracellular Cl^- was replaced with either bromide (Br^-), glutamate (Glut), iodide (I^-), isethionate (Iseth), or nitrate (NO_3^-), the magnitude of the shift in reversal potential of the macroscopic current suggested the following selectivity sequence: $NO_3^- > Br^- \geq Cl^- \geq I^- >$ Iseth $\geq$ Glut. This information was used to investigate the role of permeant ions in rectification of this current. Consistent with previous observations, when the concentration of intracellular Cl^- (Cl_i^-) was less than the concentration of extracellular Cl^- (Cl_o^-) (40 mM Cl_i^-/150 mM Cl_o^-) the current exhibited outward rectification, but when Cl_i^- was increased to equal that outside (150 Cl_i^-/150 Cl_o^-), the current no longer rectified. Rectification in the presence of asymmetrical concentrations of permeant ions on either side of the membrane is predicted by constant field theory, as described by the Goldman-Hodgkin-Katz current equation. However, when the Cl^- gradient was reversed (150 Cl_i^-/40 Cl_o^-) the current did not rectify in the opposite direction, and in the presence of lower symmetrical concentrations of Cl^- inside and out (40 Cl_i^-/40 Cl_o^-), outward rectification did not disappear. Reducing Cl_i^- by equimolar replacement with glutamate caused a concentration dependent increase in the degree of rectification. However, when Cl_i^- was replaced with more permeant anions (NO_3^- and Br^-), rectification was not observed. These results can be explained by a single binding site model based on Eyring rate theory, indicating that rectification is a function of the concentration and the permeability of the anions in the intracellular solution.

INTRODUCTION

Historically, anion conductance pathways have been overshadowed by the more ubiquitous cation conductance pathways. This has been true for the ionic currents involved in shaping the cardiac action potential (Noble, 1984). Although a role for Cl^- has been suggested (Hutter and Noble, 1961; Carmeliet, 1961), the involvement of a Cl^- conductance has been questioned (Kenyon and Gibbons, 1977, 1979).

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Recently, interest in cardiac Cl^- channels has been rekindled by the discovery of several, potentially separate, Cl^- conductances in cardiac myocytes. These include an autonomically regulated Cl^- current found in guinea-pig and rabbit ventricular myocytes (Harvey and Hume, 1989*a*, *b*; Bahinski, Nairn, Greengard, and Gadsby, 1989); a Ca^{2+}-activated Cl^- current found in rabbit atrial and ventricular myocytes (Zygmunt and Gibbons, 1991, 1992); a Cl^- current activated by swelling in canine cardiac cells (Sorota, 1992; Tseng, 1992); a Cl^- current activated by protein kinase C in guinea-pig ventricular myocytes (Walsh, 1991; Walsh and Long, 1992); and a Cl^- current activated by purinergic agonists in guinea-pig atrial cells (Matsuura and Ehara, 1992). To completely appreciate the role of Cl^- channels in the regulation of cardiac function, it will be important to fully describe the biophysical characteristics of the channels activated through each of the different pathways described above. This will help determine whether each pathway activates a unique Cl^- channel and what effect activation of each channel will have on membrane potential.

The autonomically regulated Cl^- current is perhaps the best studied of these Cl^- currents at present. It is a time-independent current that is elicited by β-adrenergic and H_2 histamine receptor stimulation via a G-protein transduced activation of adenylate cyclase, production of cAMP, and activation of protein kinase A (Harvey and Hume, 1989*a*, *b*; Harvey, Clark, and Hume, 1990; Harvey and Hume, 1990; Harvey, Jurevicius, and Hume, 1991; Bahinski et al., 1989; Hwang, Horie, Nairn, and Gadsby, 1992; Horie, Hwang, and Gadsby, 1992; Tareen, Ono, Noma, Ehara, 1991; Matsuoka, Ehara, and Noma, 1990; for review see Hume and Harvey, 1991). Because the Cl^- equilibrium potential (E_{Cl}) is normally positive to the resting membrane potential, activation of this Cl^- current would be expected to cause depolarization of the resting membrane potential. When investigated using the whole cell configuration of the patch clamp technique, activation of this Cl^- current under presumed physiological conditions (low intracellular Cl^-/high extracellular Cl^-) has been shown to play an important role in the regulation of action potential duration (Harvey and Hume, 1989*a*; Harvey et al., 1990; Harvey and Hume, 1990). However, it had little or no effect on resting membrane potential. This may be explained by the fact that under these conditions, the macroscopic current is outwardly rectifying, which reduces the magnitude of the Cl^- conductance at membrane potentials negative to E_{Cl}. Previous studies have shown that this rectification is affected by changing the intracellular Cl^- concentration (Harvey et al., 1990; Harvey and Hume, 1989*a*; Bahinski et al., 1989). In the presence of high, symmetrical concentrations of Cl^- on either side of the membrane (150 mM), the current-voltage relationship is more linear when measured over the same range. Furthermore, activation of this current under these conditions results in a much more pronounced depolarization of the resting membrane potential, due in part to the loss of rectification (Harvey et al., 1990). Therefore, understanding the mechanism of rectification will provide insight into the role this current plays in regulating the resting membrane potential in cardiac myocytes.

The Goldman-Hodgkin-Katz (GHK) current equation predicts ionic gradient-dependent rectification similar to that shown by the cAMP-regulated Cl^- current. However, it is possible that rectification could also be due to a voltage-dependent interaction of the channel with less permeant ions. It is equally possible that

rectification may be caused by competition between permeant ions at saturating binding site(s), as predicted by Eyring rate theory. Therefore, the purpose of the present study was to examine the selectivity of this Cl^- channel for various anions, and to use this information to help identify the mechanism(s) responsible for rectification of the macroscopic current. Preliminary results from these studies have been reported previously (Overholt and Harvey, 1992; Harvey, Hobert, and Overholt, 1992).

MATERIALS AND METHODS

Cell Isolation

Cells were isolated as described previously (Harvey et al., 1990), with slight modification. Briefly, hearts were rapidly excised from anesthetized, adult guinea pigs of either sex. Hearts were retrogradely perfused with Krebs-Henseleit solution that contained (in mM): NaCl (120), KCl (4.8), $CaCl_2$ (1.5), $MgSO_4$ (2.2), NaH_2PO_4 (1.2), $NaHCO_3$ (25.0) and glucose (11). The pH of the perfusate was maintained at 7.35 by equilibration with 95% O_2/5% CO_2 at 37°C. Perfusion was continued for 5 min and then switched to a nominally Ca^{2+}-free solution (Krebs-Henseleit without $CaCl_2$). After 5 min, 45 mg of collagenase B (Boehringer Mannheim, Indianapolis, IN) was added to 75 ml of the Ca^{2+}-free solution. In some preparations, 0.3 to 1 U/ml of protease type XIV (Sigma Chemical Co., St. Louis, MO) was also added to the solution. After 45 min of digestion, the right ventricle was removed, cut into small (2 × 2 mm) pieces and then incubated in the same collagenase containing solution for an additional 5–15 min. At the end of the second digestion, cells were rinsed free of collagenase and then stored in Ca^{2+}-containing Krebs-Henseleit solution. Single cells were obtained by gentle trituration and used within 8 h of preparation.

Voltage Clamp Technique

Membrane currents were recorded from isolated myocytes using the whole cell configuration of the patch clamp technique (Hamill, Marty, Neher, Sakmann, and Sigworth, 1981). Pipettes were made from borosilicate glass capillary tubing (Corning 7052, Garner Glass, Claremont, CA), and had resistances of 1-2 MΩ when filled with intracellular solution. The bath was grounded with a 3 M KCl-agar bridge to prevent changes in the junction potential between the bridge and the solution in the bath when the extracellular Cl^- concentration was reduced. Junction potential changes were <1.5 mV and were not compensated for. Currents were recorded using an Axopatch 200 voltage clamp amplifier (Axon Instruments, Foster City, CA), filtered at 5 kHz, and sampled at a frequency of 6.7 kHz using an IBM compatible computer with a TL-1-125 interface and pCLAMP software (Axon Instruments).

Solutions

Cells were dialyzed with a control intracellular solution that consisted of (in mM): CsCl (130), TEA-Cl (20), MgATP (5), EGTA (5), TRIS/GTP (0.1), and HEPES (5); pH was adjusted to 7.2 using CsOH. The control extracellular solution consisted of (in mM): NaCl (140), CsCl (5.4), $CaCl_2$ (2.5), $MgCl_2$ (0.5), HEPES (5.5), and glucose (11); pH was adjusted to 7.4 using NaOH. To determine ionic selectivity, NaCl in the extracellular solution was replaced with an equimolar concentration of the Na-salt of the replacement anion. In other experiments, unless otherwise specified in the text, Cl^- was reduced by equimolar replacement with glutamate so that the total concentration of Cl^- and glutamate was maintained at 150 mM. In some experiments, intracellular CsCl was replaced by an equiosmolar concentration of sucrose.

The cAMP-dependent Cl^- current was activated by exposing the cells to maximally stimulating concentrations of isoproterenol (ISO; 1 μM) or 3-isobutyl-1-methylxanthine (IBMX; 500 μM) in the extracellular solution. In all experiments, K^+ currents were eliminated by using K^+-free intra and extracellular solutions containing Cs^+ and/or TEA. High threshold Ca^{2+} currents were blocked by adding 1 μM nisoldipine to the extracellular solution. Low threshold Ca^{2+} channels and Na^+ channels were inactivated by using a holding potential of −30 mV. All experiments were conducted at room temperature.

Data Analysis

Current-voltage (I-V) relationships were constructed from 100 ms voltage steps from −30 mV to test potentials over the indicated ranges. No series resistance compensation was used. The Cl^- (or anion) current was defined as the agonist induced difference current determined by subtracting currents recorded in the absence of drug from currents recorded in the presence of drug. Current at each potential was measured as the average current over a 15-ms span at the end of the 100-ms step. Changes in leak current and seal resistance were monitored by examining control currents after washout of agonist. Data were not used if the amount of leak current changed during the time course of an experiment. By convention, positive current corresponds to the net outward movement of positive charge (inward movement of Cl^-) and negative current corresponds to the net inward movement of positive charge (outward movement of Cl^-). Current reversal potentials (E_{rev}) were determined by linear regression of the I-V relationships near E_{rev}. Slope conductance was determined from a linear regression of the I-V relation of outward currents.

Currents were analyzed using pCLAMP software (Axon Instruments). In some figures, current measurements were normalized to cell capacitance as determined by integration of the capacity transient elicited by a 10-mV voltage step in the whole cell configuration. All values are reported as mean ± SE. Statistical significance was determined by a one way analysis of variance and Tukey HSD test or by an unpaired *t*-test using Statgraphics software (STSC, Inc., Houston, TX). Differences in means were considered significant for $P < 0.05$. Where indicated, data were fitted to the appropriate equations using a nonlinear, least squares curve fitting routine (Sigma Plot, Jandel Corp., San Rafael, CA).

RESULTS

Anion Selectivity

To determine the relative permeability of different ions in the isoproterenol-activated (ISO-activated) Cl^- channel in cardiac myocytes, E_{rev} was determined before and after replacement of Cl^- in the external solution with a test anion. The shift of the reversal potential (ΔE_{rev}) after partial replacement of Cl^- was used to calculate the permeability of the replacement ion relative to Cl^- using the Goldman-Hodgkin-Katz voltage equation:

$$\Delta E_{Rev} = \frac{RT}{F} \ln \frac{P_{Cl}[Cl]_c}{P_{Cl}[Cl]_t + P_A[A]_o} \quad (1)$$

where R, T, and F have their usual thermodynamic meanings, P_{Cl} and P_A refer to the relative permeabilities of Cl^- ($P_{Cl} = 1$) 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 solution.

Examples of the effects of partially replacing extracellular Cl^- on membrane current in cells dialyzed with an internal solution containing 150 mM Cl^- are illustrated in Fig. 1. In *A*, after activation of the Cl^- current with 1 μM ISO, 140 of the 150 mM Cl_o^- was replaced with glutamate. It is clear that replacement of Cl^- with

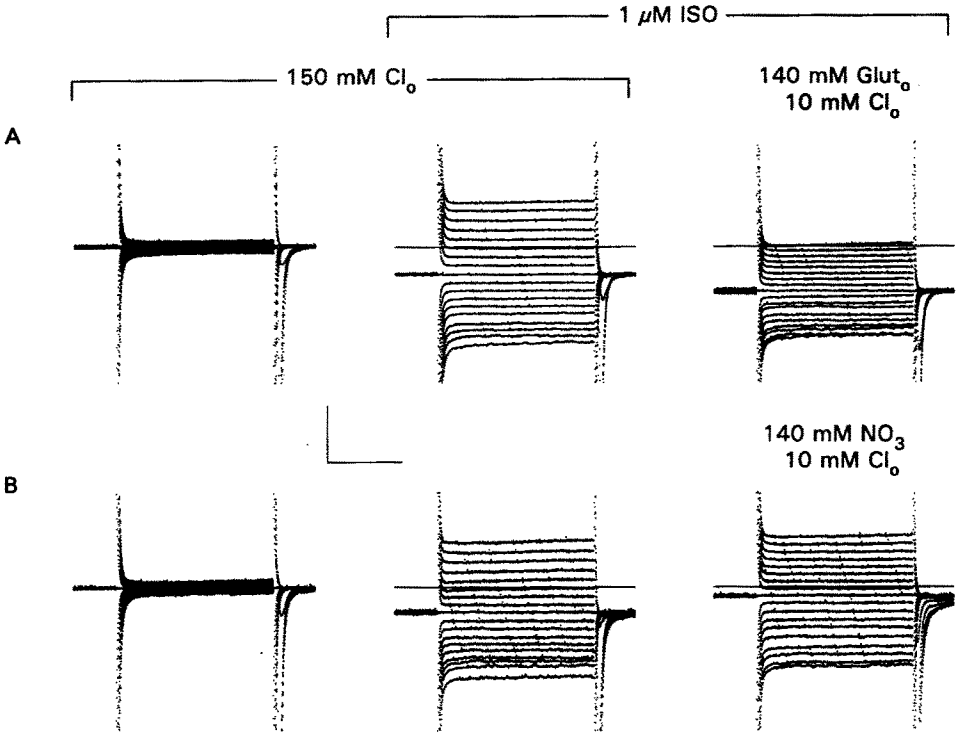

FIGURE 1. Membrane current recorded before and after partial replacement of extracellular Cl^- with various anions. Currents were elicited by 100 ms voltage clamp steps to membrane potentials between −120 and +50 mV, in 10 mV increments (holding potential −30 mV). The Cl^- current was activated by exposure to 1 μM ISO. Cells were dialyzed with an intracellular solution containing 150 mM Cl^-. For each set of traces, a horizontal line indicates the zero current level. (*A*) Currents recorded in the presence of 150 mM extracellular Cl^- (Cl_o^-) before (*left*) and after (*center*) exposure to 1 μM ISO, and currents recorded in the presence of ISO before (*center*) and after (*right*) replacement of 140 of the 150 mM Cl_o^- with an equal concentration of glutamate (*Glut*). (*B*) Currents recorded in the presence of 150 mM extracellular Cl^- (Cl_o^-) before (*left*) and after (*center*) exposure to 1 μM ISO, and currents recorded in the presence of ISO before (*center*) and after (*right*) replacement of 140 of the 150 mM Cl_o^- with an equal concentration of nitrate (NO_3^-). Calibration: (*A*) 700 pA, 50 ms; (*B*) 450 pA, 50 ms.

this anion resulted in a shift in the reversal potential to more positive potentials, indicating that the channel is less permeable to glutamate. Replacing Cl^- with glutamate also decreased the membrane conductance over the same voltage range. When Cl_o^- was replaced with NO_3^- (*B*), there was a shift of the reversal potential to

more negative membrane potentials, indicating that the channel is more permeable to NO_3^- than to Cl^-. However, replacing Cl^- with NO_3^- did not significantly affect the conductance.

The I-V relationships of the ISO induced difference currents recorded in the presence of the various external anions are shown in Fig. 2. In the presence of 150 mM Cl^- inside and out, the reversal potential of the current was near 0 mV (Fig. 2, *A*), the predicted equilibrium potential for Cl^-. When the extracellular solution was changed from one containing 150 Cl^- to one containing 10 mM Cl^- and 140

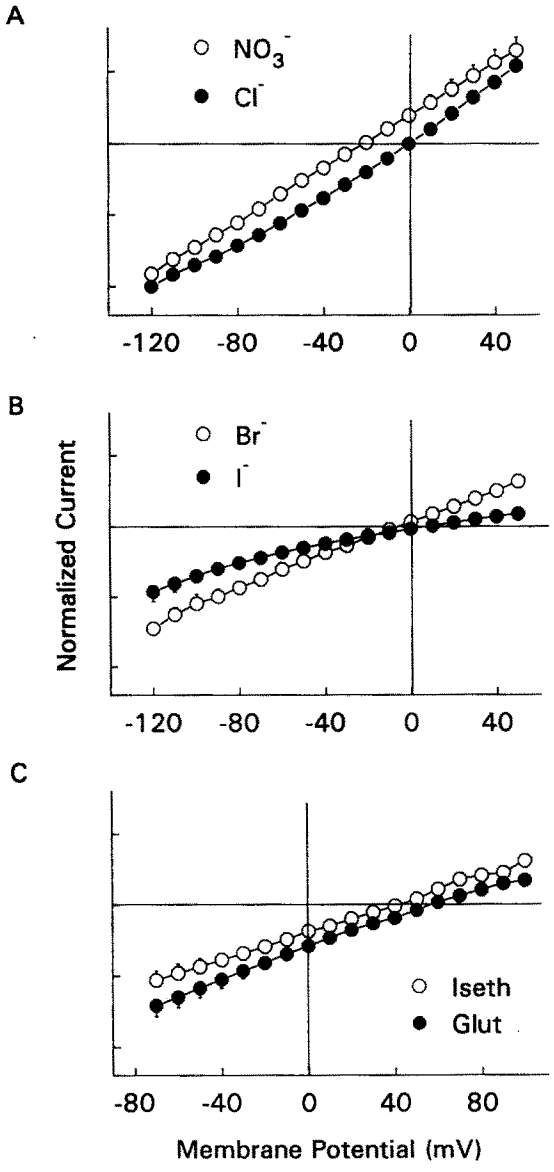

FIGURE 2. Voltage dependence of the ISO-activated Cl^- current recorded in the presence of various extracellular anions. The anion current was defined as the difference current, which was obtained by subtracting currents recorded in the absence of agonist from those recorded in its presence. Each cell was exposed to extracellular solutions containing 150 mM Cl^- and 10 mM Cl^- plus 140 mM test anion both in the presence and absence of isoproterenol. Current measurements were normalized to the maximum inward current measured in 150 mM Cl_o^-. Cells were dialyzed with an intracellular solution containing 150 mM Cl^-. (*A*) Current voltage relationships of ISO-induced current obtained before and after replacement of Cl_o^- with NO_3^- ($n = 4$). (*B*) Current voltage relationships of ISO-induced current recorded following replacement of Cl_o^- with Br^- ($n = 4$) or I^- ($n = 5$). (*C*) Current voltage relationships of ISO-induced current recorded after replacement of Cl_o^- with isethionate (Iseth; $n = 5$) or glutamate (Glut; $n = 4$). Data points represent mean ± SE.

mM NO_3^- or Br^- (Fig. 2, *A* and *B*) the reversal potential shifted in a negative direction, indicating that these ions are more permeant than Cl^-. Replacement of extracellular Cl^- with I^-, isethionate, or glutamate, on the other hand, caused a positive shift in the reversal potential (Fig. 2, *B* and *C*), indicating that they are less permeant than Cl^-. The magnitude of the shift in reversal potential and the relative permeability for each anion tested is listed in Table I. The selectivity sequence for these anions was $NO_3^- > Br^- \geq Cl^- \geq I^- >$ Iseth $\geq$ Glut. Table I also shows the effect of the replacement ions on the slope conductance of the ISO induced current. All replacement ions, except NO_3^-, decreased the slope of the I-V relationship relative to Cl^-. The conductance sequence of the current in the presence of the different anions was $NO_3^- = Cl^- >$ Glut $= Br^- \geq$ Iseth $> I^-$.

TABLE I

The Effects of the Test Anions in the Extracellular Solution on the ISO Induced Difference Currents

Anion	RP shift *mV*	Relative permeability	Relative conductance
NO_3^-	−17.9 ± 2.6	2.14 ± 0.25	1.02 ± 0.04
Br^-	−6.0 ± 2.2	1.30 ± 0.14	0.60 ± 0.10
Cl^-	0.0	1.0	1.0
I^-	+2.8 ± 1.2	0.88 ± 0.05	0.31 ± 0.02
Iseth	+45.4 ± 2.8	0.10 ± 0.02	0.50 ± 0.07
Glut	+57.6 ± 1.7	0.03 ± 0.01	0.62 ± 0.06

The shift of the current reversal potential (*RP shift*) was determined when the anion concentration in the extracellular solution was changed from 150 mM Cl^- to 10 mM Cl^- and 140 mM of the indicated test anion. The relative permeability was determined for the various test anions using the values for the RP shift in Eq. 1. Relative conductance was determined from the slope conductance of outward currents.

The Dependence of Rectification on the Ionic Gradient

Original studies of the ISO-activated Cl^- current in cardiac myocytes found rectification characteristic of that predicted by the GHK current equation (Harvey and Hume, 1989*a*; Bahinski et al., 1989; Harvey et al., 1990; Hwang et al., 1992). Models of rectification based on constant field theory, such as the GHK current equation, predict that asymmetrical concentrations of a permeant ion across a membrane should result in current that is a nonlinear function of voltage (Goldman, 1943; Hodgkin and Katz, 1949), where the degree of rectification is determined by the ratio of the slope conductances of the inward and outward current. The ionic gradient dependence of the ISO induced Cl^- current is illustrated in Fig. 3, which shows the I-V relationships from experiments where the external solution contained 150 mM Cl^- and cells were dialyzed with an internal solution that contained either 40 (n = 9) or 150 mM Cl^- (n = 9). Intracellular Cl^- was reduced by equimolar replacement with glutamate. The data are reasonably well described by the GHK current equation:

$$I = P_{Cl} z^2 \frac{V_m F^2}{RT} \frac{[Cl]_i - [Cl]_o \exp\left(\frac{-zFV_m}{RT}\right)}{1 - \exp\left(\frac{-zFV_m}{RT}\right)} \quad (2)$$

where I is the experimentally measured current, V_m is membrane potential, and $[Cl]_i$ and $[Cl]_o$ are the intra- and extracellular concentrations of Cl^-, respectively. Data were fitted by adjusting the value of P_{Cl}. However, the experimentally obtained values rectified slightly more than predicted. This included a small degree of rectification observed with 150 mM Cl^- inside the cell. Despite the quantitative differences, the experimental results are qualitatively similar to those predicted by the GHK current equation.

To more rigorously test the hypothesis that the behavior of this Cl^- current could be predicted by constant field theory, the effects of changing the Cl^- concentration gradient on rectification were examined under more extreme conditions. The GHK current equation predicts that reversal of the Cl^- gradient (high Cl_i^-/low Cl_o^-) should result in a current that rectifies in the inward direction. Fig. 4 *A* displays examples of raw currents from a single cell dialyzed with a solution containing 150 mM Cl^- and

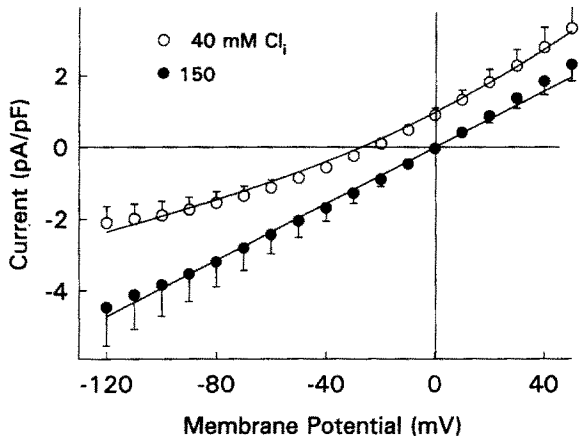

FIGURE 3. Effect of changing the intracellular Cl^- concentration on the voltage dependence of the ISO-activated Cl^- current. Cells were bathed in an extracellular solution containing 150 mM Cl^- and dialyzed with an intracellular solution containing either 150 (n = 9) or 40 mM Cl^- (n = 9); glutamate was used as the replacement anion. Isoproterenol (1 μM) induced difference currents were normalized to cell capacitance. Solid lines represent best fit to data using the GHK current equation, as described in the text. Data points represent mean ± SE.

with external solutions containing 40 or 150 mM Cl^-. The average (n = 8) I-V relationship for the difference currents is shown in Fig. 4 *B*. Reversal of the Cl^- gradient resulted in a positive shift of E_{rev} (31.9 ± 1.6 mV) as expected. However, the current did not exhibit inward rectification. The data obtained when Cl_o^- was reduced to 40 mM were fit using Eq. 2. It is clear that under these conditions, the current does not behave as predicted by constant field theory.

Currents were also recorded from cells dialyzed with an intracellular solution containing a low concentration of Cl^- (40 mM), a condition previously shown to cause outward rectification. Under these conditions, the GHK current equation predicts that changing Cl_o^- from high (150 mM) to low (40 mM) should linearize the I-V relationship. Fig. 5 *A* shows examples of raw currents recorded from a single cell dialyzed with 40 mM Cl^- and exposed to external solutions containing 150 and 40 mM Cl^-. The average (n = 9) I-V relationship for the difference currents is shown in

Fig. 5 *B*. Changing the external solution from high to low Cl^- caused the expected shift in E_{rev}, but more importantly, it did not eliminate outward rectification. The data obtained when external Cl^- was reduced to 40 mM were fit to Eq. 2, but again there was a poor correlation between the measured values and those predicted by constant field theory. Based on these findings, rectification of the cAMP-dependent Cl^- current cannot be attributed to the concentration gradient of Cl^- across the

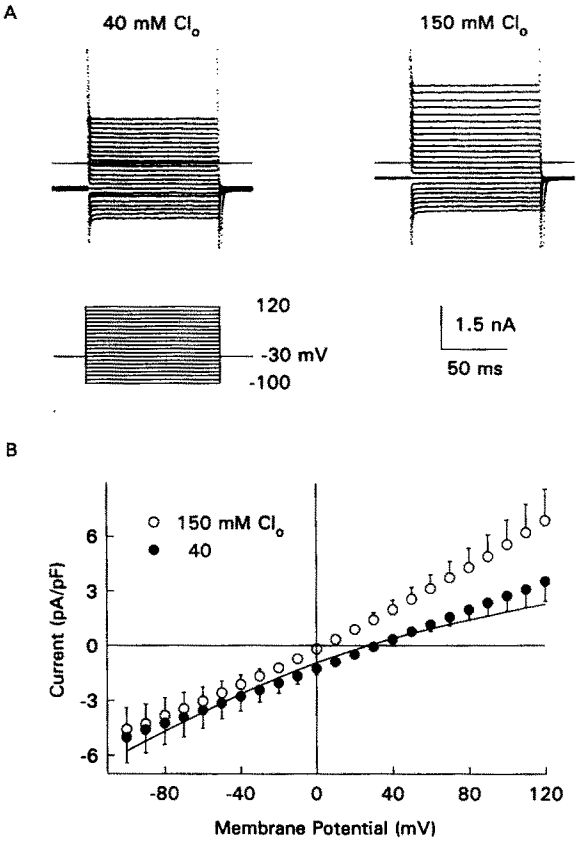

FIGURE 4. Effect of changing the extracellular Cl^- concentration on the voltage dependence of IBMX-activated Cl^- current in the presence of high intracellular Cl^- concentrations. Cells were dialyzed with an intracellular solution that contained 150 mM Cl^- and the extracellular solution was changed from one containing 150 mM Cl^- to one containing 40 mM Cl^- (glutamate used as a replacement anion). (*A*) Examples of unsubtracted current traces recorded from a cell after activation of the Cl^- current with 500 μM IBMX in the presence of 40 mM Cl^- (*left*) and after switching to an external solution containing 150 mM Cl^- (*right*). Currents were elicited using the voltage clamp protocol shown. Zero current level is indicated by horizontal lines. (*B*) Voltage dependence of the IBMX induced difference currents. Each cell was exposed to external solutions that contained 40 and 150 mM Cl^-. Data points represent mean ± SE (n = 8) of measurements normalized to cell capacitance. Solid line represents relationship predicted by the GHK current equation for 150 mM Cl_i^- and 40 mM Cl_o^-.

membrane, rather, it appears to be associated with the concentration of Cl^- in the intracellular solution.

Chloride Dependence of Rectification

To better characterize the Cl^- dependence of rectification, the effects of varying internal and external Cl^- were further investigated. Partial replacement of external

Cl^- with glutamate significantly reduced the conductance of both the inward and outward current. In cells dialyzed with 40, 80, 120, and 150 mM Cl_i^-, the slope conductance of the outward current recorded in the presence of 40 mM Cl_o^- was 70 ± 3% (n = 9), 59 ± 5% (n = 6), 61 ± 3% (n = 5) and 69 ± 4% (n = 8), respectively, of that measured in the presence of 150 mM Cl_o^-. If reducing Cl_o^- does not affect rectification, then it should reduce the magnitude of the conductance of the inward and outward current similarly. To facilitate comparison of the effects of changing

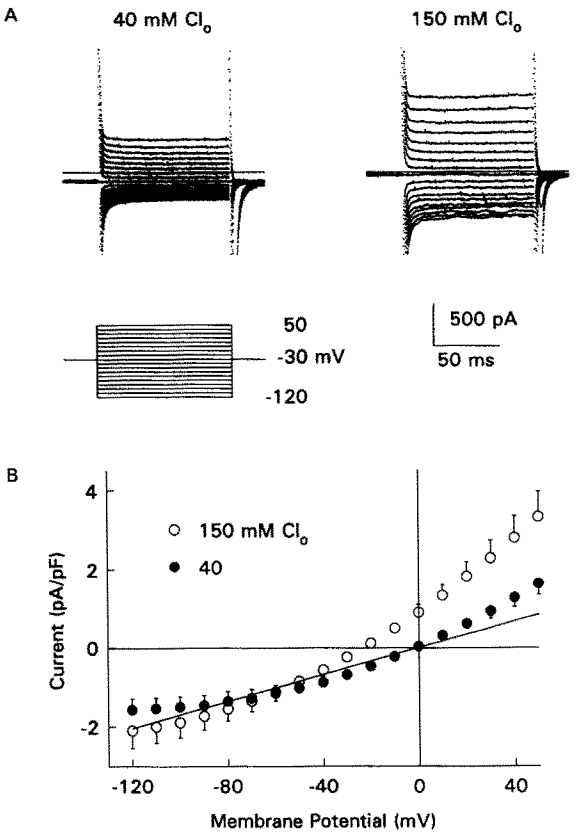

FIGURE 5. Effect of changing the extracellular Cl^- concentration on the voltage dependence of the ISO-activated Cl^- current in the presence of a low intracellular Cl^- concentration. Cells were dialyzed with an intracellular solution that contained 40 mM Cl^- and the extracellular solution was changed from one containing 150 mM Cl^- to one containing 40 mM Cl^- (glutamate used as a replacement anion). (*A*) Examples of unsubtracted current traces recorded from a cell following activation of the Cl^- current with 1 μM ISO in the presence of 40 mM Cl^- (*left*) and after switching to an external solution containing 150 mM Cl^- (*right*). Currents were elicited using the voltage clamp protocol shown. Zero current level is indicated by horizontal lines. (*B*) Voltage dependence of the ISO induced difference currents. Each cell was exposed to external solutions that contained 40 and 150 mM Cl^-. Data points represent mean ± SE (n = 9) of measurements normalized to cell capacitance. Solid line represents the relationship predicted by the GHK current equation for 40 mM Cl_i^- and 40 mM Cl_o^-.

Cl_o^- on the relationship between inward and outward currents (i.e., the shape of the I-V relationships), data were normalized to the value of the current recorded 30 mV positive to the reversal potential and plotted as a function of driving force. Fig. 6 *A* summarizes the effect of changing Cl_o^- from 150 to 40 mM on the I-V relationships over a range of different intracellular Cl^- concentrations. Once currents are normalized, the I-V relationships in the presence of 40 and 150 mM Cl_o^- are superimpos-

able, and it becomes apparent that Cl_o^- has no effect on rectification at any intracellular Cl^- concentration tested.

The effect of varying the intracellular Cl^- concentration on the behavior of the current was also investigated. The slope conductance of the outward current varied between the groups of cells dialyzed with different intracellular Cl^- solutions. In cells dialyzed with 20, 40, 80, 120, and 150 mM Cl^-, the slope conductance of the outward

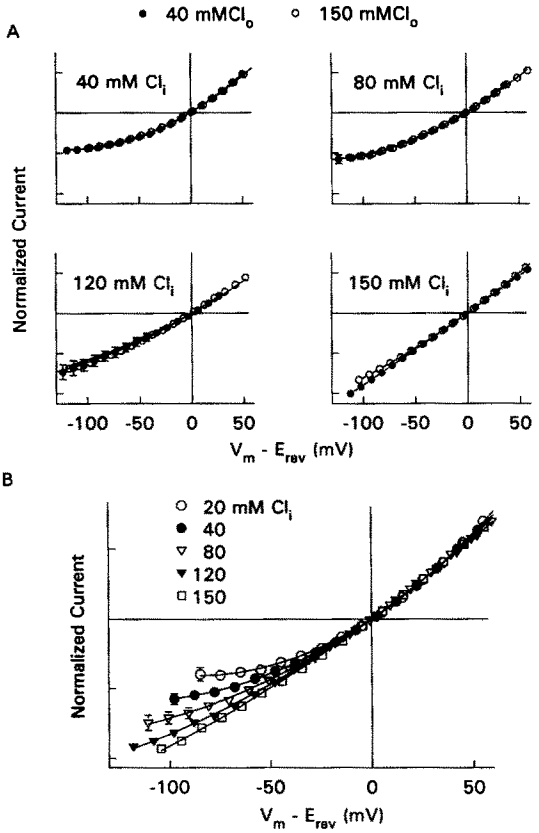

FIGURE 6. Intracellular Cl^- dependence of rectification. Changes in the relationship between inward and outward current were investigated by plotting current measurements normalized to the estimated outward currents at 30 mV positive to the reversal potential as a function of driving force ($V_m - E_{rev}$). (*A*) Effect of varying extracellular Cl^- on the relationship between inward and outward current in cells dialyzed with intracellular solutions which contained 40 ($n = 9$), 80 ($n = 6$), 120 ($n = 5$), or 150 ($n = 8$) mM Cl^-. Cells were dialyzed with only one intracellular Cl^- concentration, but each cell was exposed to extracellular solutions which contained 40 and 150 mM Cl^-. (*B*) Effect of varying intracellular Cl^- on the relationship between inward and outward current in cells bathed in 150 mM Cl^-. Same data as in *A* with the addition of experiments in which cells were dialyzed with an internal solution containing 20 mM Cl^- ($n = 5$). Each set of symbols represents currents recorded from a separate group of cells. Lines represent best fit of data points by second order polynomial. Because currents were plotted as a function of driving force, actual current measurements at E_{rev} + 30 mV were not always available. Therefore, currents at that potential were estimated by interpolation using the polynomial fit. Data points represent mean ± SE.

current was 50 ± 12, 43 ± 8, 40 ± 8, 27 ± 5, and 41 ± 4 S/F, respectively (not shown). There is no apparent pattern with respect to an effect of intracellular Cl^- on the density of the outward current, and, using ANOVA and the Tukey HSD, there were no significant differences found between groups ($P > 0.05$). Fig. 6 *B* compares the I-V relationships of normalized currents in cells dialyzed with various internal Cl^- concentrations when the external solution contained 150 mM Cl^-. Cells were

dialyzed with a solution containing either 20 (n = 5), 40 (n = 9), 80 (n = 6), 120 (n = 5) or 150 (n = 8) mM Cl^-. Unlike Cl_o^-, changing Cl_i^- did have a significant effect on the relationship between inward and outward current. Fig. 6 *B* shows that rectification becomes stronger as internal Cl^- is decreased, especially at more negative potentials. Put another way, the degree of outward rectification is inversely related to the internal Cl^- concentration. Moreover, the effects of changing Cl_i^- and Cl_o^- demonstrate that rectification is dependent on the intracellular Cl^- concentration, not on the concentration gradient per se.

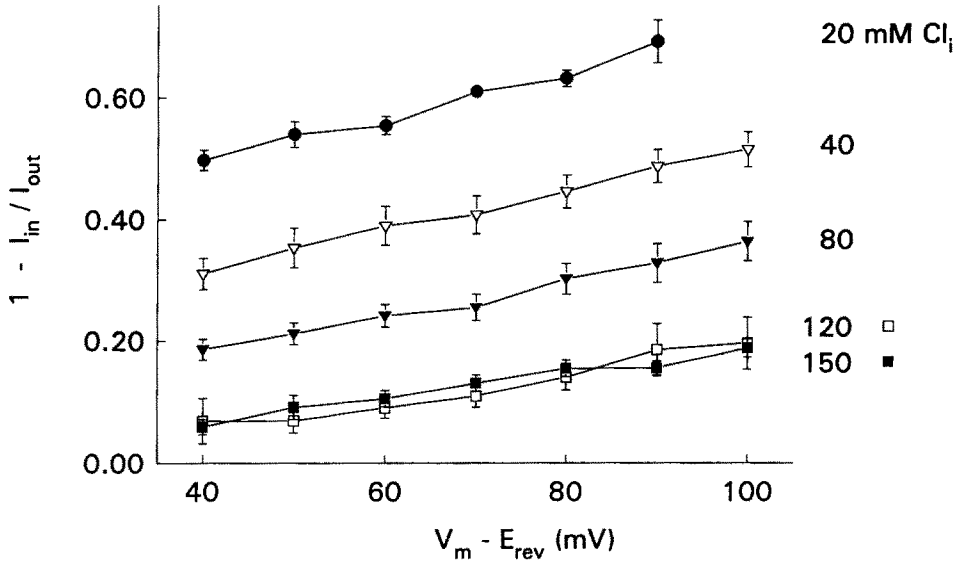

FIGURE 7. The voltage and concentration dependence of rectification of ISO-induced Cl^- current in cells dialyzed with various intracellular Cl^- concentrations. The difference in the magnitude of inward and outward current relative to the outward current measured at the same absolute driving force was determined at various concentrations of internal Cl^-. The relative decrease in inward current ($1 - I_{in}/I_{out}$) is shown as a function of driving force ($V_m - E_{rev}$) for different Cl_i^- concentrations. Separate groups of cells were dialyzed with internal solutions containing different Cl^- concentrations. All measurements were made in the presence of 150 mM external Cl^-. In some cases it was necessary to estimate the corresponding outward current at more positive potentials by extrapolation. This was done by linear regression of the experimentally measured data points at potentials positive to the reversal potential. Data points represent mean ± SE.

The relationship between inward and outward current at different internal Cl^- concentrations is shown more clearly in Fig. 7. This figure shows rectification, measured as the decrease in inward current relative to the outward current at a given driving force ($1 - I_{in}/I_{out}$), plotted as a function of driving force for different internal Cl^- concentrations. It can be seen that rectification increases in a voltage and concentration dependent manner. Decreasing the concentration of internal Cl^-, or increasing the driving force at a given Cl_i^-, increases the difference between the inward and outward current produced by the same absolute driving force. Further-

more, the nonzero slope of the individual relationships indicates that rectification also depends on voltage. However, the voltage dependence appears to be independent of Cl_i^-.

The Effect of Replacement Anion Permeability on Rectification

The previous results suggest that rectification could be inversely related to the intracellular Cl^- concentration: increasing Cl_i^- decreases rectification by increasing

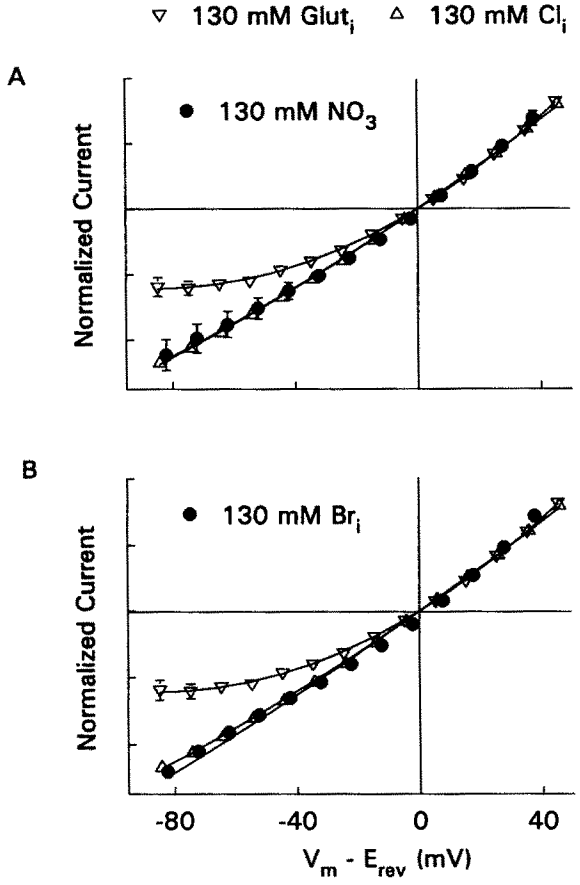

FIGURE 8. Voltage dependence of the ISO-activated Cl^- current recorded in the presence of various intracellular anions. Anion currents were activated with 1 μM isoproterenol. Changes in the relationship between inward and outward current were determined by plotting the normalized difference current as a function of driving force ($V_m - E_{rev}$). Cells were dialyzed with intracellular solutions containing either 150 mM Cl^- or 20 mM Cl^- plus 130 mM test anion. The extracellular solution contained 150 mM Cl^-. (*A*) Voltage dependence of currents recorded in cells dialyzed with 150 mM Cl^-, 20 mM Cl^- plus 130 mM glutamate, or 20 mM Cl^- plus 130 mM NO_3^- ($n = 5$). (*B*) Voltage dependence of normalized currents recorded in cells dialyzed with 150 mM Cl^-, 20 mM Cl^- plus 130 mM glutamate, or 20 mM Cl^- plus 130 mM Br^- ($n = 2$). Data representing cells dialyzed with 150 mM Cl^- ($n = 8$) and 20 mM Cl^- plus 130 mM gluta-mate ($n = 5$) are the same in both panels. Lines represent best fit of data points by second order polynomial. Data points represent mean ± SE.

the relative amount of inward current independent of the concentration gradient. Alternatively, rectification could depend on the intracellular concentration of the relatively impermeant replacement ion, glutamate, because increasing the intracellular glutamate concentration increases the degree of rectification. Taken together, these results suggest that rectification may be related to the permeability of the anions present in the intracellular solution. To test this hypothesis, cells were

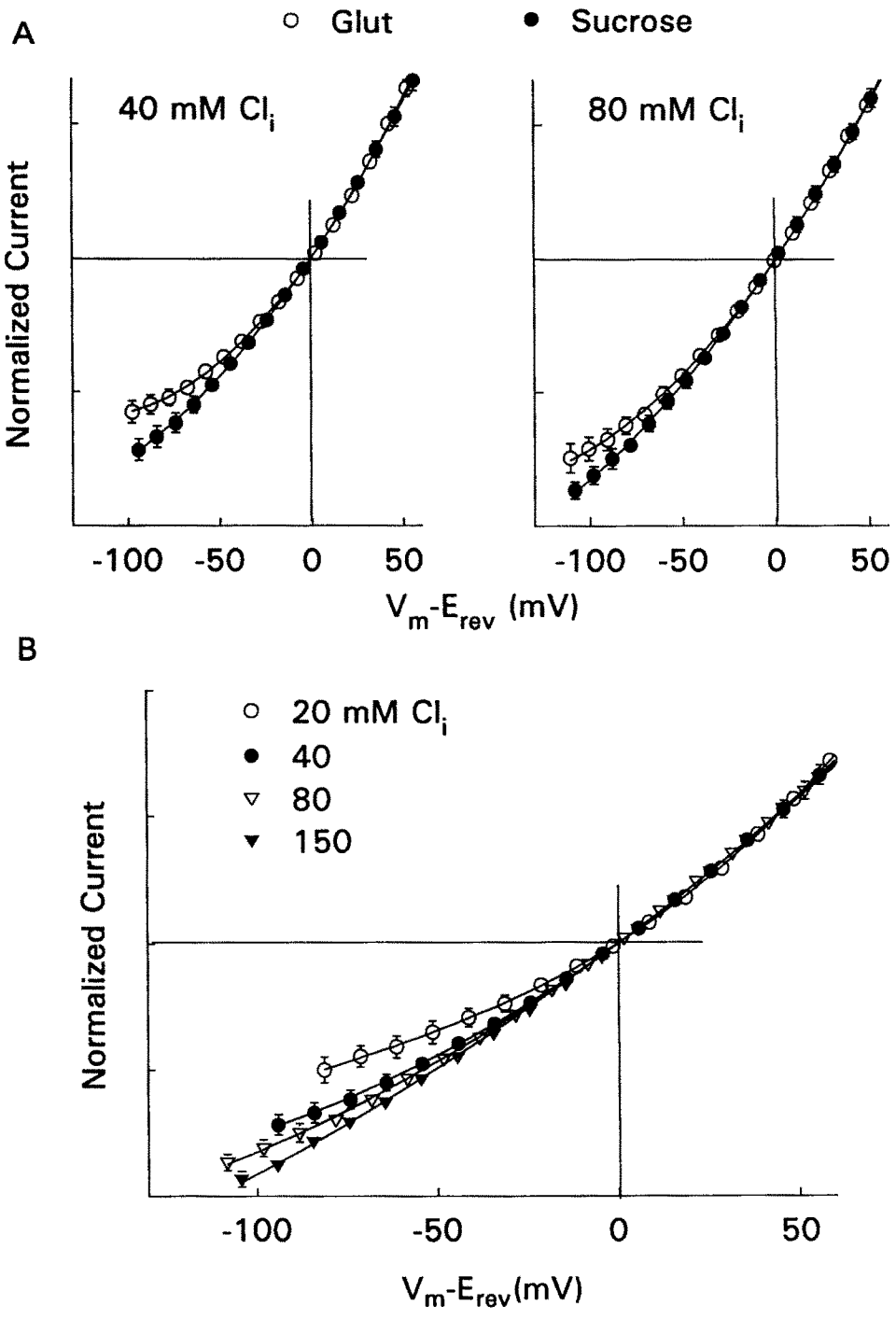
A
Glut
Sucrose
40 mM Cl_i
80 mM Cl_i
Normalized Current
-100 -50 0 50
-100 -50 0 50
V_m-E_{rev} (mV)
B
20 mM Cl_i
40
80
150
Normalized Current
-100 -50 0 50
V_m-E_{rev}(mV)

FIGURE 9.

dialyzed with internal solutions in which 130 mM Cl_i^- was replaced with an equimolar concentration of NO_3^- or Br^-, anions that are more permeant than glutamate. Fig. 8 shows the results of these experiments. The I-V relationship of the difference current obtained from cells dialyzed with 20 mM Cl_i^- and 130 mM NO_3^- ($n = 5$) is superimposable with the I-V relationship of the current obtained from cells dialyzed with 150 mM Cl_i^-. A similar result was obtained when cells were dialyzed with 20 mM Cl_i^- and 130 mM Br^- ($n = 2$). Because NO_3^- and Br^-, anions at least as permeant in the channel as Cl^-, supported a relative inward current magnitude similar to Cl^-, rectification in glutamate containing intracellular solutions may be due to the presence of this relatively impermeant anion.

The Role of Impermeant Anions in Rectification

The data in Fig. 8 suggest that rectification may be due to the permeability of the replacement ion, such as glutamate, in the intracellular solution. Therefore, it is possible that in the absence of glutamate in the intracellular solution, the current would not exhibit rectification regardless of the Cl^- concentration. Therefore, to determine whether Cl_i^- alone can affect rectification, currents were recorded from cells dialyzed with internal solutions in which Cl^- was reduced without replacement by another anion. To accomplish this, CsCl was replaced with an osmotically equivalent concentration of sucrose. This bulky, uncharged molecule should have no effect on any site(s) that anions interact with.

Fig. 9 *A* shows the effect of sucrose substitution in cells dialyzed with 40 ($n = 6$) and 80 ($n = 5$) mM Cl_i^-. In both cases, rectification of the current recorded from cells dialyzed with sucrose containing solutions is less than that recorded from cells dialyzed with glutamate containing solutions. The difference between the normalized inward currents recorded when Cl_i^- was replaced with sucrose and glutamate is significant at all driving potentials between -40 and -100 mV in cells dialyzed with 40 mM Cl^-. Therefore, glutamate itself appears to be responsible for at least part of the reduction of inward current. However, the current still exhibits outward rectification in the absence of glutamate. This can be seen more clearly in Fig. 9 *B*, which shows normalized I-V relationships at four different levels of Cl_i^- in the absence of glutamate. Assuming sucrose has no effect, this figure illustrates the effect of Cl_i^- alone on rectification. Again, increasing Cl_i^- causes an increase in the relative inward

FIGURE 9. (*opposite*) Intracellular Cl^- dependence of rectification in the absence of replacement anion. Anion currents were activated with 1 μM isoproterenol. Changes in the relationship between inward and outward current were determined by plotting the normalized difference current as a function of driving force ($V_m - E_{rev}$). (*A*) Currents recorded in cells dialyzed with intracellular solutions containing 40 mM Cl^- (*left*) using either Cs-glutamate ($n = 9$) or sucrose ($n = 6$) to replace CsCl, and 80 mM Cl^- (*right*) using either Cs-glutamate ($n = 6$) or sucrose ($n = 5$) to replace CsCl. (*B*) Comparison of currents measured in cells dialyzed with 20 ($n = 6$), 40, 80, and 150 mM intracellular Cl^- when sucrose was used to replace CsCl. Data representing cells dialyzed with sucrose containing solutions are the same in both panels. The extracellular solution contained 150 mM Cl^- in all experiments. Lines represent best fit of data points by second order polynomial. Data points represent mean ± SE.

current at more negative potentials. As in Fig. 6 *A*, the I-V relationships with sucrose containing solutions are superimposable when Cl_o^- is changed from 150 to 40 mM (not shown). Therefore, the effect produced by changes in Cl_i^- in the absence of glutamate is also independent of the concentration gradient. These figures also show that glutamate, while not solely responsible for rectification, does modulate the effect of Cl_i^- on inward current.

The relationship between intracellular Cl^- and rectification in the absence of glutamate is shown in Fig. 10 *A*. When I-V relationships are normalized with respect

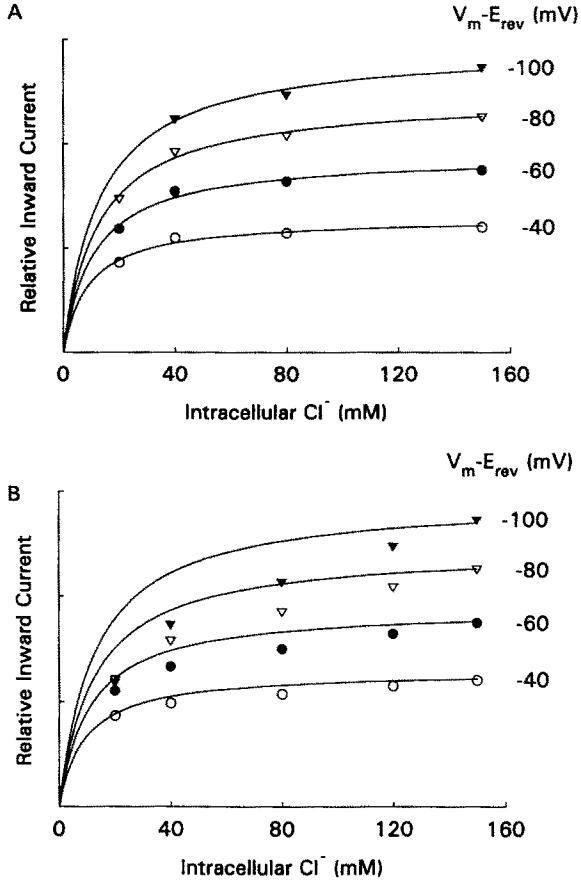

FIGURE 10. Dependence of the relative inward current on intracellular Cl^-. The relative inward current was obtained from plots of the normalized currents versus driving force (Figs. 6 and 9). Symbols represent absolute values for inward currents calculated by interpolation using the polynomial fit to the experimental data. (*A*) Intracellular Cl^- dependence of the relative inward current in experiments where the Cl^- concentration was varied by replacing CsCl with sucrose. Lines represent the best fit of the data to Eq. 3. Estimated values for K_m and I_{max} are listed in Table II. (*B*) Intracellular Cl^- dependence of the relative inward current in experiments where the Cl^- concentration was varied by replacing CsCl with Cs-glutamate. Relationship to data obtained when no anion was used to replace Cl^- is indicated by the solid lines, which are the fits of data shown in *A*.

to the outward current (see Figs. 6 *B* and 9 *B*), the effect of changing intracellular Cl^- on rectification, or the relationship between the inward and outward current, can be assessed by plotting the magnitude of the relative inward current as a function of intracellular Cl^- concentration. Assuming that Cl_i^- is acting at a specific binding site, the magnitude of the relative inward current should saturate as Cl_i^- is increased. The relationship between Cl_i^- and current can therefore be expressed in the form of the

TABLE II

The Dependence of Relative Inward Current on Cl_i^- Can Be Expressed in the Form of the Michaelis-Menten Relation (Eq. 3)

	Driving force			
	mV			
	−40	−60	−80	−100
I_{max}	−1.29	−1.89	−2.45	−2.94
K_m (Cl^-)	9.05	10.89	12.5	12.92
K_I (Glut)	592.22	266.02	152.28	86.95

The data from Fig. 10 A were fit by this relation to obtain the values for I_{max} and K_m at the indicated driving force. The effect of glutamate on this relationship can be explained by competitive inhibition by glutamate as expressed in Eq. 4. The K_I for glutamate was determined by fitting the data from Fig. 10 *B* to Eq. 4 using the values for I_{max} and K_m calculated above.

Michaelis-Menten equation:

$$I_{rel} = \frac{I_{max}[Cl]_i}{K_m + [Cl]_i}, \tag{3}$$

where $[Cl]_i$ is the intracellular Cl^- concentration, I_{rel} is the magnitude of the relative inward current at a given driving force, I_{max} is the magnitude of the maximum relative inward current at a given potential, and K_m is the Cl^- concentration at which

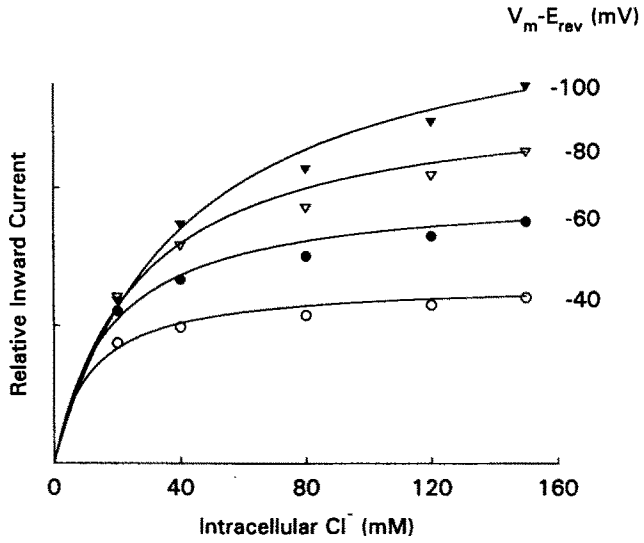

FIGURE 11. Relative inward current dependence on intracellular Cl^- and glutamate. Rectification in the presence of glutamate was greater at all Cl^- concentrations, suggesting that glutamate competes with Cl^- for a common binding site. Data in Fig. 10 *B* were fit using Eq. 4. Values for K_m and I_{max} were constrained to the values estimated from the fit in Fig. 10 *A* and were used to obtain the fit shown in this figure by adjusting the K_I value for glutamate. Estimated values for K_I are listed in Table II.

relative inward current is 50 percent of I_{max}. Fig. 10 *A* shows that the experimental data can be fit well by this relationship. The resulting values for I_{max} and K_m are listed in Table II. At all driving forces, increasing Cl_i^- increases the amount of relative inward current.

Fig. 10 *B* illustrates the relationship between Cl_i^- and the normalized inward current in the presence of glutamate. The data points were obtained from experiments in which intracellular Cl^- was varied by replacement with glutamate (data from experiments illustrated in Fig. 6 *B*). In this case the data were fit using the same parameters derived from the fit to the data in *A*. The Michaelis-Menten relation for Cl^- alone does not fit the experimental data obtained in the presence of glutamate. The data points are shifted to the right, suggesting that glutamate increases the apparent K_m for Cl^-. This is consistent with what would be expected if glutamate is acting as a competitive antagonist, in which case the effect of Cl^- in the presence of glutamate should be described by:

$$I_{rel} = \frac{I_{max}[Cl]_i}{[Cl]_i + K_m\left(1 + \frac{[Glut]_i}{K_I}\right)} \quad (4)$$

where $[Glut]_i$ is the concentration of glutamate in the intracellular solution and K_I is the inhibition constant for the effect of glutamate. Using the values for I_{max} and K_m calculated for Cl^- in the absence of glutamate, the new equation can be fit to the experimental data obtained in the presence of glutamate to calculate a value for K_I at different potentials. Fig. 11 illustrates that the experimental data are well fit by Eq. 4. The resulting values for K_I are also shown in Table II. Intracellular glutamate therefore appears to compete with Cl^- for a common binding site, thereby increasing the apparent K_m for Cl^-.

DISCUSSION

In the present study it was shown that changing Cl_i^-, while maintaining Cl_o^- at 150 mM, affects rectification in a manner that can be described by constant field theory; rectification appears to be associated with asymmetrical concentrations of permeant ions on either side of the membrane (Fig. 3). However, constant field theory cannot explain the absence of rectification when the gradient is reversed (Fig. 4), nor the presence of rectification with low, symmetrical Cl^- concentrations (Fig. 5). Furthermore, the strength of rectification is inversely related to the intracellular Cl^- concentration, regardless of the extracellular Cl^- concentration (Fig. 6). One explanation for this behavior could be that rectification is actually caused by glutamate, the relatively impermeant anion used to replace Cl^-. This is supported by the fact that rectification is not observed when Cl_i^- is replaced with more permeant ions (NO_3^- or Br^-). However, when Cl_i^- is reduced without replacement by another anion, the current still exhibits rectification (Fig. 9 *B*), but the degree of rectification observed at a given Cl_i^- concentration is greater in the presence of glutamate. These results can be explained by Cl^- and glutamate acting at a common, saturable, binding site accessible from the intracellular surface of the membrane.

Permeation of the Cl^- Channel

The effect produced by changing intra- and extracellular anion concentrations on the whole cell ISO-activated Cl^- current may be viewed as an indirect measure of the unitary channel conductance, because the macroscopic current is time-independent. Although effects of Cl^- and other anions on channel gating behavior cannot be ruled out, for this to be the case, activation or deactivation of the current under rectifying conditions would have to be extremely fast. Furthermore, other studies have suggested that this channel does not exhibit voltage dependent gating. Ehara and Ishihara (1990) reported that the single channel current voltage relationship from the β-adrenergically activated Cl^- channel in guinea pig ventricular myocytes exhibited rectification similar to that of the whole cell current. More importantly, there was no variation in open probability with voltage. If it is assumed that the macroscopic current does reflect the unitary conductance, then the results of the present study clearly demonstrate that permeation of the ISO-activated Cl^- channel in cardiac muscle cannot be described by constant field theory for the reasons delineated above.

An alternative approach that can be used to explain permeation through an ion channel is based on Eyring rate theory (Hille, 1975; Eisenman and Horn, 1983), where passage of an ion through a channel is viewed as involving a series of discrete steps through energetically favorable and less favorable states. According to this theory, relative permeabilities depend mainly on the heights of the barriers in the energy profile, whereas conductance is determined by the depth of the energy wells (Hille, 1975). Because different factors determine permeability and conductance, this may explain why the relative conductance of the current measured in the presence of different anions does not parallel the relative permeability (see Table I). The selectivity sequence for the ISO-activated Cl^- channel is $NO_3^- > Br^- \geq Cl^- \geq I^- >$ Iseth $\geq$ Glut. This is identical to sequence 3 identified by Wright and Diamond (1977), which arises from a moderately weak anion-site interaction. The fact that rate theory predicts saturation of current as the concentration of permeating ions is increased may explain the variations in conductance of the current measured in the presence of different anions. This, of course, assumes that changing the concentration of anions does not indirectly affect the conductance by perturbing the cAMP regulatory pathway (for example, see Walsh and Long, 1992).

Rate Theory Model For Rectification

Our results indicate that intracellular Cl^- regulates the degree of rectification in a concentration dependent manner, which suggests that Cl^- is acting at a specific binding site (see Fig. 10*A*). The data also indicate that the effects of Cl^- and glutamate are voltage dependent, suggesting that they are acting at a site within the membrane electric field. Further, the fact that rectification also appears to be related to the permeability of the anions in the intracellular solution (Fig. 8), and that permeant ions can compete for this site (Fig. 11), suggests that the site is located in the channel pore. This type of behavior can be described by rate theory. Using this approach, the simplest model is to view the channel as having a single binding site

within the pore, in which case the process of permeation is described by:

$$A_o + X \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} AX \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} A_i + X$$

where A_o and A_i represent the permeant anion at the extra- and intracellular surface of the membrane, respectively; X is the anion binding site found within the channel; and k_1, k_{-1}, k_2, and k_{-2} are the rate constants to and from the binding site. The rate constants represent the relative heights of the energy barriers leading to and from the binding site. Rectification can then be explained by several factors: competition between anions of different permeability; the voltage dependence of the individual rate constants (determined by the position of the barrier in the membrane electrical field); and asymmetric barrier heights at the internal and external face of the membrane. We tested the possibility that a single binding site model could explain the characteristics of rectification of the ISO-activated Cl^- current using the method described in detail by Hille (1975) and further characterized by Eisenman and Horn (1983). For our simulations, the position of the internal binding site was estimated from the current ratios determined in the presence and absence of glutamate (from Fig. 9*A*) using the method of Woodhull (1973). The electrical distance from the internal surface was estimated to be between .47 and .41 (for 40 and 80 mM Cl_i^-, respectively). The rate constants and electrical distances to the peaks of the barriers for Cl^- and glutamate were determined by fitting the model to the experimental data and are indicated in the legend for Fig. 12.

Fig. 12 shows that rate theory can account for the salient characteristics of the macroscopic current. Like Fig. 3, *A* shows that the model predicts rectification and a reversal potential shift when internal Cl^- is reduced from 150 to 40 mM by replacement with glutamate. Like Fig. 6, *B* shows that reducing extracellular Cl^- from 150 to 40 mM by replacement with glutamate has little effect on rectification. *C* and *D* show that currents simulated by the model also closely approximate the experimental data obtained in the presence and absence of glutamate, respectively. These panels show that the model predicts Cl^--dependent rectification, and, at any given concentration of intracellular Cl^-, rectification is greater in the presence of glutamate (compare Fig. 12, *C* with *D*). It is therefore possible to predict rectification that depends on Cl_i^- but not Cl_o^-, and to describe the voltage dependence of the macroscopic currents using a single binding site model with one set of parameters. However, the possibility that this channel has multiple binding sites cannot be ruled out.

Another possibility is that rectification observed in the absence of glutamate is due to the presence of some other less permeant anion(s) in the intracellular solution. One candidate for such an effect is HEPES. This anionic pH buffer has been shown to block Cl^- channels in other preparations (Hanrahan and Tabcharani, 1990). However, in the same preparation, TRIS was without effect, and in guinea pig ventricular myocytes, replacing HEPES with TRIS does not affect the degree of rectification (Overholt and Harvey, unpublished observation). Another possible candidate is free ATP, although it may be difficult to assess its effects on rectification,

because it is an obligatory component for activation of this current, and, therefore, cannot be simply removed or replaced.

These results are consistent with the idea that intracellular Cl^- modulates the relative conductance of the inward current, while reducing extracellular Cl^- de-

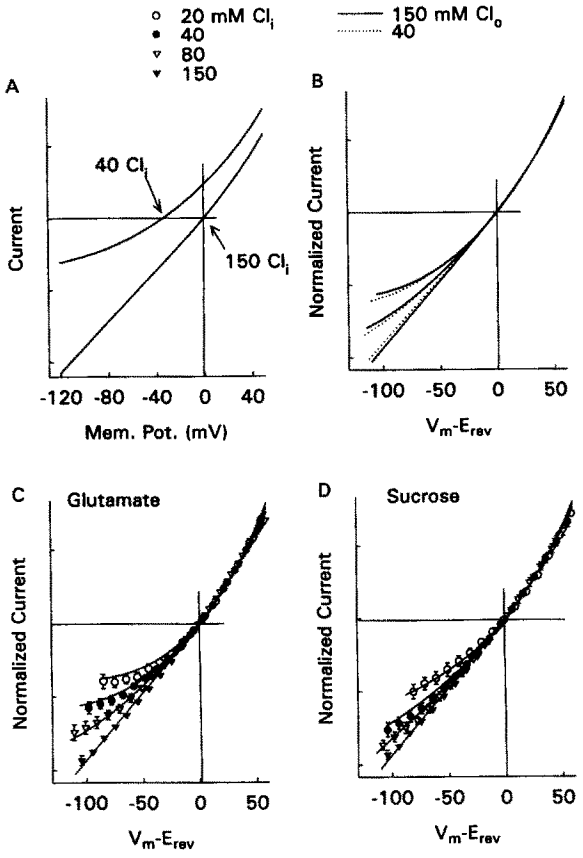

FIGURE 12. Predictions of a one-site, two-barrier model based on Eyring rate theory. Current values were calculated using a single set of parameters. The relative values for the rate constants at 0 mV were: for chloride k_1 = 4.2, k_{-1} = 258.06, k_2 = 190.48, and k_{-2} = 3.1; for glutamate k_1 = 0.0001, k_{-1} = 0.225, k_2 = 40, and k_{-2} = 0.018. The channel was modeled as having asymmetric barriers, such that each rate constant was affected by the following fraction of the membrane field: k_1, 0.30; k_{-1}, 0.27; k_2, 0.36; k_{-2}, 0.07. (*A*) Effect of replacing intracellular Cl^- with glutamate on non-normalized currents as in Fig. 3. Current voltage relationships predicted by the model with 150 mM Cl_i^-/150 mM Cl_o^- (E_{rev} = 0 mV) and 40 mM Cl_i^- 150 mM Cl_o^- (E_{rev} = −34.5 mV). (*B*) The effect of reducing extracellular Cl^- from 150 (*solid line*) to 40 mM (*broken line*) by equimolar replacement with glutamate on model currents normalized as in Fig. 6 *A* when Cl_i^- was 40, 80, and 150 mM (glutamate replacement). (*C*) Dependence of rectification on intracellular Cl^- and glutamate. Data generated by the model (*solid lines*) were normalized the same as the experimental data from Fig. 6 *B*. Currents were simulated in the presence of 20, 40, 80, and 150 mM Cl_i^- (glutamate replacement, 150 mM Cl_o^-). (*D*) Dependence of rectification on intracellular Cl^- in the absence of anion replacement. Data generated by the model (*solid lines*) were normalized the same as the experimental data from Fig. 9 *B*. Currents were simulated in the presence of 20, 40, 80, and 150 mM Cl_i^- (150 mM Cl_o^-).

creases the conductance of both the inward and outward anion currents equally. This behavior is also predicted by the rate theory model (Fig. 12 *B*). The effect of changing the intracellular Cl^- concentration on the absolute magnitude of either the inward or outward current, on the other hand, was not determined experimentally,

because this would have required changing the intracellular Cl^- concentration in the same cell. From the present results, comparisons could only be made between groups of cells dialyzed with different Cl^- concentrations. Although the data, normalized to cell capacitance, suggests that changing the intracellular Cl^- concentration does not affect the conductance of the outward current, there appears to be a great deal of variability in current density between cells (Takano and Noma, 1992). The rate theory model predicts that altering Cl_i^- affects the conductance of both inward and outward current, but a greater affect on inward current is responsible for the Cl^- dependence of rectification.

Relationship to Other Cl^- Channels

It is now generally accepted that the cystic fibrosis transmembrane regulator (CFTR) codes for a Cl^- channel. This channel is found in the secretory epithelia of many tissues and mutations in the amino acid sequence are responsible for the genetic disease cystic fibrosis. CFTR shares many functional characteristics with the ISO-activated Cl^- channel in cardiac myocytes. The CFTR Cl^- channel is activated by cAMP, the current conducted through this channel exhibits outward rectification in the presence of asymmetrical (low inside/high outside) Cl^- concentrations, and the current is not blocked by disulfonic acid derivatives (Anderson, Gregory, Thompson, Souza, Paul, Mulligan, Smith, and Welsh, 1991; Berger, Anderson, Gregory, Thompson, Howard, Maurer, Mulligan, Smith, and Welsh, 1991; Anderson and Welsh, 1991; Cliff and Frizzell, 1990; Harvey, 1993; Nagel, Hwang, Nastiuk, Nairn, and Gadsby, 1992). Recent molecular data has also demonstrated that there is a high degree of sequence homology between CFTR and an ISO-activated Cl^- channel cloned from cardiac myocytes (Levesque, Hart, Hume, Kenyon, and Horowitz, 1992; Horowitz, Tsung, Levesque, Hart, Hume, 1993). This is consistent with the findings of the present study, which show that the selectivity sequence of the ISO-activated Cl^- channel in cardiac myocytes is the same as that for CFTR: $Br^- > Cl^- > I^-$ (Anderson et al., 1991). However, our results differ from another report that found I^- to be more permeant than Cl^- in a cAMP-dependent Cl^- channel in cardiac myocytes (Walsh and Long, 1992).

Recent studies have also identified a number of different Cl^- conductance pathways in cardiac tissue. The channels responsible for these currents have not been clearly distinguished, and at present it is possible that one or more may arise from dual regulation of the same channel. Tseng (1992) recently identified an outwardly rectifying, time and voltage independent Cl^- current in canine ventricular myocytes induced by cell swelling. This current differs from the ISO-activated current in that aspartate, but not glutamate, caused a positive shift in the reversal potential. This current was also found to be insensitive to inhibition of protein kinase, suggesting that phosphorylation is not involved in its regulation.

A time and voltage independent Cl^- current activated by protein kinase C (PKC) has also been reported in guinea pig ventricular myocytes (Walsh, 1991). However, this current differs from the ISO-activated Cl^- current described in the present study, in that the PKC activated current did not rectify even with low intracellular Cl^- concentrations. The selectivity sequence and the characteristics of rectification of the ISO-activated Cl^- current therefore suggest that it is conducted by a channel that is different from the swelling and PKC activated Cl^- channels.

Physiological Implications

The experiments in this study have shown that rectification of the ISO-activated Cl^- current in guinea pig ventricular myocytes depends on the concentration and permeability of the anions inside the cell. How then, would one expect this current to behave in-vivo? Our findings would suggest that, with a low intracellular Cl^- concentration, between 10 and 24 mM (Baumgarten and Duncan, 1987), and with a relatively high concentration of less permeant amino acids (Fozzard and Arnsdorf, 1992), it seems probable that rectification is a characteristic of the ISO-activated Cl^- current under physiological conditions. However, changes in rectification may be a mechanism contributing to aberrant electrophysiological properties of cardiac muscle under pathological conditions. Increases in intracellular Cl^- during ischemia (Ramasamy, Zhao, Gitomer, Sherry, Malloy, 1992) may result in a Cl^- conductance that no longer rectifies, which could lead to spontaneous depolarizations in ventricular muscle (Harvey et al., 1990), causing disturbances in the normal sequence of tissue activation. A recent study has also shown significant changes in the concentration of both glutamate and aspartate in response to a regulatory volume decrease associated with a hyposmotic challenge (Rasmusson, Davis, and Lieberman, 1993). Further, the sensitivity of the inward current to changes in Cl_i^- suggests that this may be a mechanism by which intracellular Cl^- concentration may be regulated. Increasing Cl_i^- would facilitate inward current, or Cl^- efflux.

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