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# Single-Channel Analysis of a Potassium Inward Rectifier in Myocytes of Newborn Rat Heart

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**Summary.** Unitary $K^+$ currents in single cells isolated from ventricular muscle of newborn rat hearts were measured in response to different potentials and $[K]_o$. The *I/V* curves were linear for potentials more negative than the zero-current voltage; especially in high $[K]_o$ (150 mM KCl), no clear outward currents could be detected indicating a drastic rectification in the inward direction. The channel is mainly selective to $K^+$ but $Na^+$ ions are also carried ($P_{Na}/P_K = 0.056$). The channel conductance is proportional to the square root of $[K]_o$ but $Na^+$ ions seem to have a facilitatory effect on $\gamma_K$, the single-channel conductance. The channel activity, measured as $P_o$, i.e. the probability to find the channel in open state, decreased as the membrane was hyperpolarized. This behavior was tentatively explained by an inactivation process as the membrane becomes more negative. The rate constants of the transitions between the different states were calculated according to a C-O-C model. A control of the gating process by permeant ion $K^+$ was postulated, based on the increase of one of the rate constants from the closed to the open state with $[K]_o$. Finally, the macroscopic *I/V* curves calculated from $P_o$ and $\Delta i$, the unit current, were found to be characteristic of a ion-blocked inward rectifier.

**Key Words** ventricular cells · newborn rats · single channel · patch clamp · potassium · inward rectification

## Introduction

Potassium currents play an important role in the resting potential and repolarization phase of the action potential and diastolic depolarization in cardiac muscle. In order to better understand the mechanism underlying these currents, extracellular patch-clamp experiments have thus been performed on several cardiac cell preparations: embryonic chick heart (DeFelice & Clapham, 1982; Clapham & DeFelice, 1984), bullfrog atrial cells (Momose et al., 1983), guinea-pig heart cells (Sakmann & Trube, 1984*a,b*), guinea-pig atrial cardioballs (Bechem et al., 1983) and rat ventricular cells (Josephson & Brown, 1984; Rousseau et al., 1984).

In some cases, the observed myocardium $K^+$ channels showed inward rectification. For some preparations, the macroscopic inward rectification was found to be caused by an increase of $P_o$, the open-channel probability, as the cell is hyperpolarized (Bechem et al., 1983) whereas in others, inward rectifying *I/V* curves were observed at the single-channel level with an increase of $P_o$ as the cell is depolarized (Josephson & Brown, 1984; Rousseau et al., 1984; Sakmann & Trube, 1984*a,b*).

Inward rectifying single channels have also been observed on several other preparations: HeLa cells (Sauvé et al., 1983), tunicate egg (Fukushima, 1981; 1982), nodal cells (Sakmann et al., 1983), and rat myotubes (Ohmori et al., 1981).

We present, in this paper, results obtained on a potassium channel recorded on freshly isolated cardiac cells of newborn rats. We found at first that this particular channel *I/V* curve shows inward rectification and secondly that the increase of $P_o$ as the cell is depolarized, is a function of the external potassium concentration. The relationship between the macroscopic *I/V* curve predicted from these channel events and that measured for K current on some excitable cells will be discussed.

## Materials and Methods

Single-channel currents were recorded from freshly isolated cardiac cells from neonatal rats (1 to 2 days) using the patch-clamp techniques as described by Neher et al. (1978) and Hamill et al. (1981).

The isolated cardiac cells were obtained by enzymatic treatment from the lower third of the heart. Small pieces of heart (about 10) were minced with scissors, placed in a 25-ml erlenmeyer flask with 3 ml of normal physiological medium (*see* composition below) plus collagenase 0.01% and shaken at 37°C in a Dubnoff shaker. A greater number of cells was obtained with a calcium and magnesium-free medium plus 0.1% collagenase. There were no morphological nor functional differences between the cells obtained by these two procedures. The shaking period lasted 15 min for each batch and the first two batches containing numerous damaged cells were discarded. The batches were

**Table.** Solutions[a]

| Solutions (mM) | Na | K | Ca | Choline | HEPES |
|---|---|---|---|---|---|
| 300 K | 0 | 300 | 0 | 0 | 5 |
| 150 K | 0 | 150 | 0 | 0 | 5 |
| 150 K-Acetate | 0 | 150 | 0 | 0 | 5 |
| 150 K-Ca | 0 | 150 | 2.2 | 0 | 5 |
| 75 K | 75 | 75 | 0 | 0 | 5 |
| 50 K | 100 | 50 | 0 | 0 | 5 |
| 20 K-ONa | 0 | 20 | 0 | 130 | 5 |
| 10.8 K | 140 | 10.8 | 0 | 0 | 5 |
| 5.4 K | 144.6 | 5.4 | 0 | 0 | 5 |

[a] Chloride salts were used except for the 150 K-acetate where potassium acetate ($CH_3COOK$) was employed. All solutions were buffered at pH 7.4 and filtered (0.2 $\mu$m pore size). Atropine (0.1 mM) was added to the choline solution.

pooled in normal medium plus 0.1% bovine serum albumin (BSA) and placed on ice. At the end of the isolation period, the cells were centrifuged at 300 × *g* for 20 min. The pellet was resuspended in normal physiological medium plus BSA 0.1% and layered in plastic petri dishes. An average of one hour was required in order to get a weak but sufficient binding of the cells. All the experiments were performed at room temperature. The bathing solution contained (mM): NaCl 130.0; KCl 5.4; $CaCl_2$ 2.2; $MgCl_2$ 0.24; HEPES 5.0, and dextrose 5.0; the pH was adjusted to 7.4. The pipettes were filled with one of the solutions listed in the Table.

The pipettes were made by pulling capillary tubes in two steps on a vertical puller. Two different kinds of glass were employed: when hematocrit capillary tubes were used, pipettes were not fire polished and the seal resistance was low (1 to 2 GΩ). With fire-polished pipettes made of Pyrex® glass (Corning 7740) the seal resistance was greater and had values ranging from 10 to 20 GΩ.

The experiments were done in cell-attached configuration where the patch of membrane is still attached to the intact cell. The recording circuit was described by Hamill et al. (1981). The currents were recorded in steady-state conditions, one minute after stepping to a new potential.

The currents were obtained with a Dagan 8900 and recorded on an FM tape recorder EMI at bandwidth of 2.5 KHz. Before recording, the signal was filtered at 600 Hz by means of two low-pass, four-pole Butterworth filters connected in series (Frequency Devices model 745 PB-3, 745 PB-5). The data were digitized at 1200 Hz (PDP 11/23) and stored on hard disks (Digital RL02). The digitized records were analyzed in segments in order to correct for a possible drift of the base line. The computer analysis consisted mainly in measuring amplitude histograms which in addition to the current amplitude gave the probability for the channel to be in the open and closed states. (A detailed description of the procedure will be published later.[1])

Kinetic analysis of the open and closed time intervals was performed according to a method proposed by Roux and Sauvé (1985).

[1] R. Sauvé, C. Simoneau, G. Bedfer, and G. Roy, 1985. Single-channel analysis of the $K^+$ permeability in HeLa cancer cells. *J. Membrane Biol. (Submitted)*

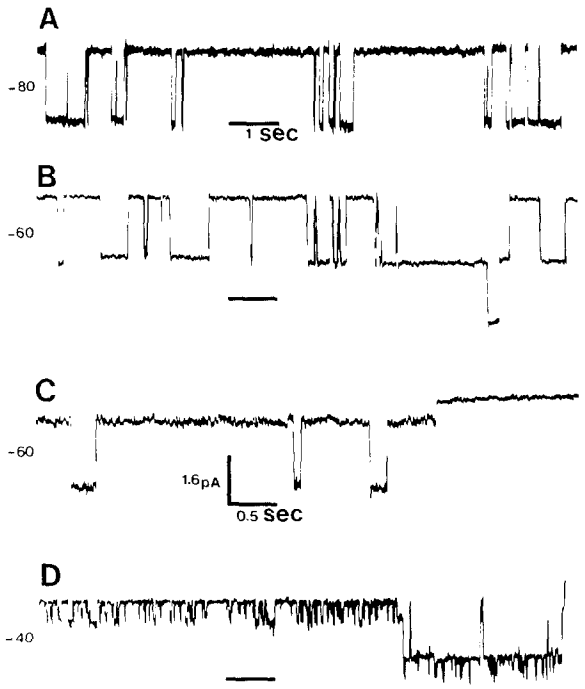


**Fig. 1.** Single-channel events recorded during a cell-attached patch experiment with a 150 mM KCl-filled pipette in (*A*). The membrane potential of the patched area is −80 mV. The channel activity is shown to occur in bursts, which sometimes appear in clusters. (*B*) Simultaneous opening of two identical channels under the patch. During our experiments we never saw more than two active channels under a patch which indicate a low channel density. Membrane potential −60 mV, 150 mM KCl-filled pipette. (*C*) Current jumps of low amplitude and long duration occasionally recorded. Membrane potential −60 mV, 150 mM KCl-filled pipette. (*D*) Current jumps of low amplitude and short duration, a current jump similar to those in *A* and *B* is also observable. Membrane potential −40 mV, 300 mM KCl-filled pipette

## Results

### Single Channel

Figure 1 (*A*, *B*, *C*) shows single-channel current recordings obtained in cells attached with a 150 mM KCl-filled pipette. The first record illustrates the current fluctuations pattern most frequently encountered; we will refer to these particular single-channel events as type I, and only this type of fluctuations will be under the scope of this paper. There was no externally applied voltage (*B*, *C*) in this case, so that the effective voltage across the patched area corresponded to the cell resting potential (−60 mV). It should be apparent from these records that channel occurred mainly in bursts separated by silent periods. Multiple events could also be observed occasionally (*B*). In some cases, these bursts appeared in clusters as already described in

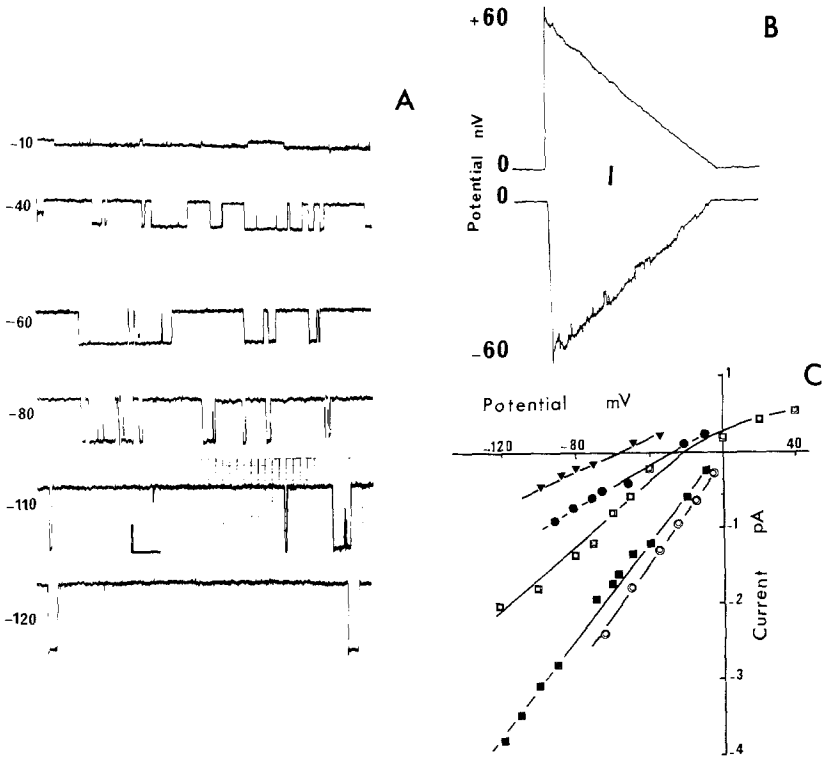


**Fig. 2.** Effect of voltage on single-channel current amplitude. (*A*) Example of single-channel recordings at six different membrane potentials for a patch pipette containing 150 mM KCl. Calibrations: horizontal, 0.5 sec; vertical 1.6 pA. (*B*) Current recorded during a voltage ramp from +60 or −60 mV to 0 mV with 150 mM KCl-filled pipette. Current jumps are only recorded for potentials more negative than the zero-current potential. Calibration: vertical, 1.6 pA. (*C*) Single-channel current voltage curves obtained from patch pipettes containing: 5.4 mM KCl + 144.6 mM NaCl (▼); 50 mM KCl + 100 mM NaCl (●); 75 mM KCl + 75 mM NaCl (□); 150 mM KCl (■); 150 mM K acetate (○)

studies on Ach-activated channels (Sakmann et al., 1980), $Ca^{2+}$-activated $K^+$ channels (Pallota et al., 1981; Maruyama et al., 1983; Moczydlowski & Latorre, 1983) and $K^+$ channels from HeLa cells (Sauvé et al. 1983) (Fig. 1*A*).

Current fluctuations of low amplitude but long duration were also recorded in about 5% of our experiments. The channel seemed to remain open for a long period of time with no clear appearance of short closures. As shown in Fig. 1*C*, both types of channels can occur simultaneously. We also observed a third class of current jumps characterized by rapid fluctuations of low amplitude; an example of single-channel recordings obtained with a 300 mM KCl-filled pipette in which this type of current jump is superimposed to fluctuations of type I is presented in Fig. 1*D*. Typical cluster arrangements did not appear in this particular case.

Single-channel currents were recorded with 150, 75, 50, 10.8 and 5.4 mM KCl-filled pipettes in which ionic force was maintained by NaCl, and with pipettes containing 300 mM KCl.

The channel *I/V* relationship was studied for each of the K concentrations considered. Clear inward current jumps, whose amplitude increased as more negative membrane potentials were applied, could in all cases be observed (Fig. 2*A*). Outward currents of lower amplitude could only be recorded with KCl concentrations equal to 75 mM or less. In fact, no outward current jump could clearly be detected with electrodes containing 150 mM KCl, even at applied voltages 60 mV higher than the zero-current potential value (Fig. 2*B*). The inward current *I/V* relationship for each of the KCl concentrations considered was found to be essentially linear, corresponding to single-channel conductances of 30, 20, 15.5, 15 and 12 pS for external medium containing 150, 75, 50, 10.8 and 5.4 mM KCl, respectively (Fig. 2*C*). Ten experiments were done with 300 mM KCl pipettes. However, the membrane seemed to deteriorate very rapidly, and workable records of approximately 1 min could be obtained in three cases only. The single-channel conductance then measured ranged from 30 to 40 pS.

## Ionic Selectivity and K Ions Effect on the Single Conductance

In order to determine the ionic selectivity of the channel, the value of the zero-current voltage was determined for various $[K]_o$. As seen from Fig. 2*C* the zero-current potential is shifted toward more negative values as $[K]_o$ is decreased. The points could not be fitted by a straight line indicating that the channel is not exclusively permeable to $K^+$ (Fig. 3*A*). As the pipettes contain $Na^+$ ions, the points were fitted by the Goldman-Hodgkin-Katz equation in which $P_{Na}/P_K$ was found to be 0.056. The substitution of $Cl^-$ by $CH_3COO^-$ in the patch electrode

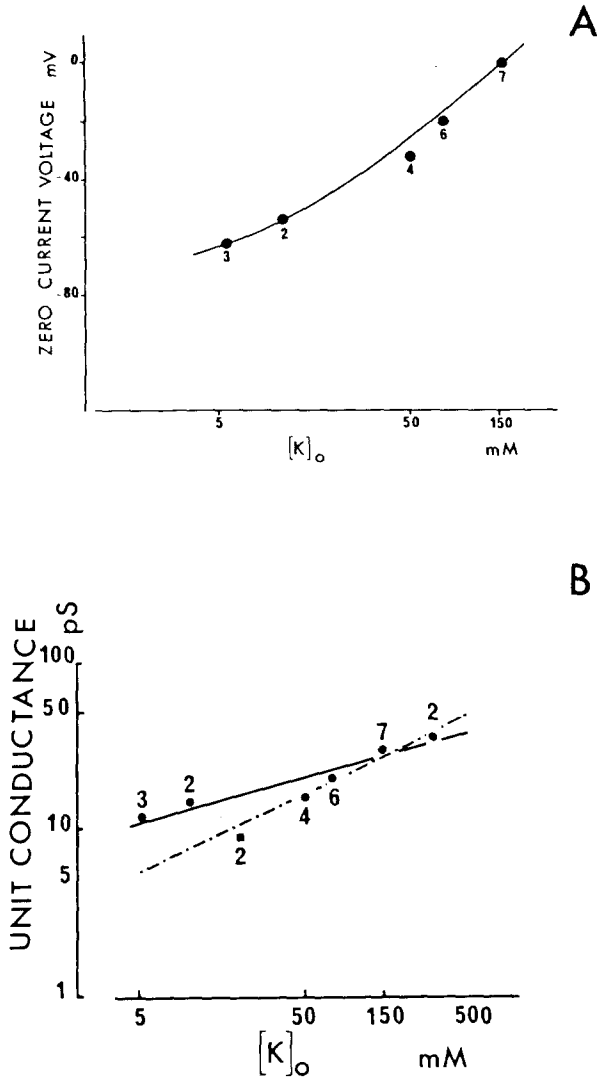


**Fig. 3.** (*A*) Zero-current potential as a function of $[K]_o$. The continuous line was fitted to the experimental points with the Goldman-Hodgkin-Katz equation with a $P_{Na}/P_K$ of 0.056. (*B*) The unit conductance $\gamma_K$ as a function of $[K]_o$. The continuous line, fitted through all the points, has a slope of 0.287. The interrupted line fitted through the points obtained for K concentrations above 50 mM has a slope of 0.46. The point (■) was obtained with a 20 mM KCl-NaCl free filled pipette in which NaCl was replaced by choline chloride plus atropine (0.1 mM). Number of experiments is indicated for each point

did not have any appreciable effect on the channel *I/V* characteristics and anion permeability of the channel may be ruled out (Fig. 2*C*). Finally, from the amplitude of the applied voltage needed for zero current with 150 mM KCl electrodes, it was concluded that the resting potential of these cells should approximately be equal to −60 mV, a value in agreement with results obtained using conventional microelectrode.

The logarithmic plot of the single unit conductance versus $[K]_o$ shows (Fig. 3*B*) that the channel conductance can be expressed as $\gamma_K \simeq [K]_o^{\alpha}$ with $\alpha = 0.287$ which is not totally equivalent to the value of 0.5 found for inward rectifying channel on other preparations (Sakmann & Trube, 1984*a*). However, if only K concentrations above 50 mM are considered, the slope increases to 0.46. Experiments were carried out with a 20 mM KCl-filled pipette in which NaCl was replaced by choline chloride plus atropine (130 mM, 0.1 mM). The conductance value obtained (9.2 pS) was less than that expected with KCl + NaCl (20, 130 mM) and in accordance with the points obtained for $[K]_o$ higher than 50 mM (Fig. 3*B*).

## Opening Probability and Macroscopic *I/V* Curve

In order to determine the effect on the channel kinetics of both the transmembrane voltage and $[K]_o$, $P_o$, the open-channel probability, was computed as a function of the applied voltage for two KCl concentrations (150, 75 mM). It should first be apparent from the records shown in Fig. 2*A*, that the channel remained closed for a longer period of time as the cell was hyperpolarized. This observation was confirmed by an analysis of the steady-state open-channel probability as a function of the membrane voltage. As illustrated in Fig. 4*A*, $P_o$ increases as more positive voltages are applied indicating that the channel is activated with depolarization. It ought to be mentioned, however, that the values we obtained for $P_o$ represent the global open-channel probability, and do not correspond to the open probability computed from the single-channel events occurring only within a burst (Methfessel & Boheim, 1982; Bechem et al., 1983). It can also be seen from Fig. 4*A*, that for a given membrane voltage $P_o$ increases as a function of the external K concentration. The observed channel kinetics is thus both transmembrane potential and external K dependent. This point is particularly important since it indicates that the open probability of this channel in normal solution (5.4 mM) should be rather small. In order to determine if the single-channel events we observed using the patch-clamp technique could eventually be related to known voltage-clamp results, the mean current taken as $P_o \cdot \Delta i$, where $\Delta i$ is the height of the current jump at a given potential, was computed for three of the KCl concentrations (150, 75, 10.8). The resulting curves are shown in Fig. 4*B*. It can be seen for instance, that the macroscopic current increases as the membrane is hyperpolarized, but decreases at high hyperpolarizations due to the voltage dependency of $P_o$.

Similar *I/V* relationships could be obtained by recording macroscopic currents in the whole cell configuration (Hamill et al., 1981). In a normal bathing solution (5.4 mM KCl), a well-clamped fast in-

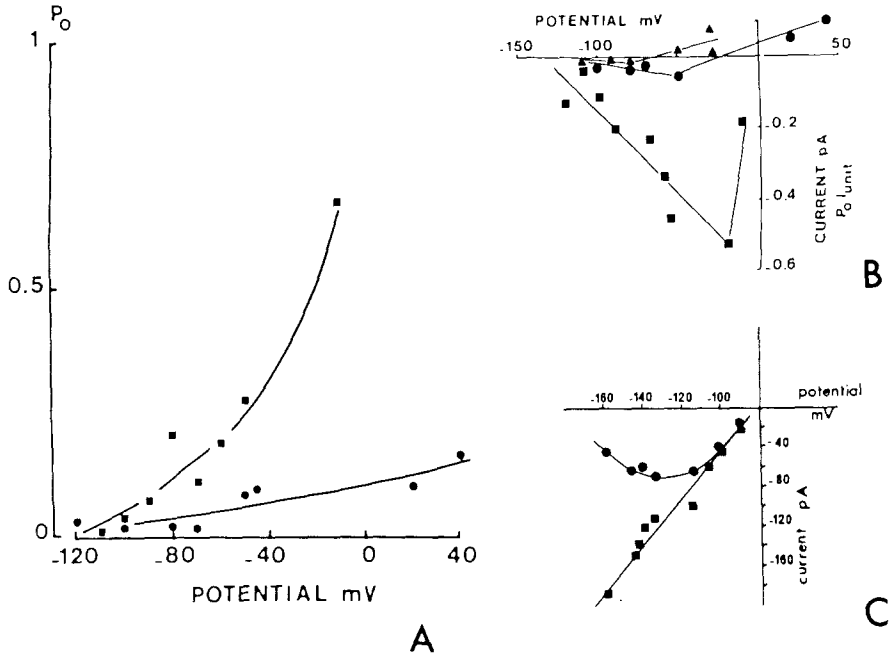


**Fig. 4.** (*A*) Effect of voltage and $[K]_o$ on the open state probability $P_o$. $P_o$ was evaluated from probability density histograms. 150 mM KCl (■), 75 mM KCl + 75 mM NaCl (●) (*B*) Voltage dependence of the steady-state mean current calculated as $P_o \cdot \Delta i$. 150 mM KCl (■), 75 mM KCl + 75 mM NaCl (●), 10.8 mM KCl + 140 mM NaCl (▲). (*C*) Macroscopic potassium current. The current was recorded in the whole cell recording configuration with a patch electrode in a McEwen bathing medium (5.4 mM KCl). Instantaneous current-voltage relationship (■) and steady-state current-voltage relationship (●). Electrode-filling solution (mM): NaCl 20; KCl 130; EGTA 5, and HEPES 5

ward current ($I_{Na}$) could be recorded following membrane depolarization from a holding potential of −80 mV (M.D. Payet, *unpublished observations*). Instantaneous and steady-state currents of different amplitudes were recorded following a hyperpolarizing pulse greater than 40 mV, from a holding potential of −80 mV. Under these conditions, an inactivation process could be observed. The instantaneous current-voltage relationship was found to be linear with a conductance of 2.3 nS. The steady-state current increased for voltages down to −130 mV and then decreased as the membrane voltage was more negative (Fig. 4*C*).

## Channel Kinetics

Gating kinetics were obtained by statistical analysis of the current fluctuations one minute after a new voltage had been applied.

For all the voltages we studied, frequency histogram analysis consistently confirmed that the channel has two closed states and just one open state (Fig. 5).

## Voltage and $[K]_o$ Dependency of Mean Lifetime

The mean open lifetime, $\tau_{open}$, the mean closed lifetimes of the short closed intervals, $\tau_{cfast}$, and of the long closed intervals, $\tau_{cslow}$, were determined from frequency histograms for different voltages in two $[K]_o$. Figure 6 (*A, B*) shows that the open and closed time constants are voltage dependent, $\tau_{open}$ increasing with depolarization while $\tau_{cslow}$ decreases. Consequently, the channel is more frequently and for a

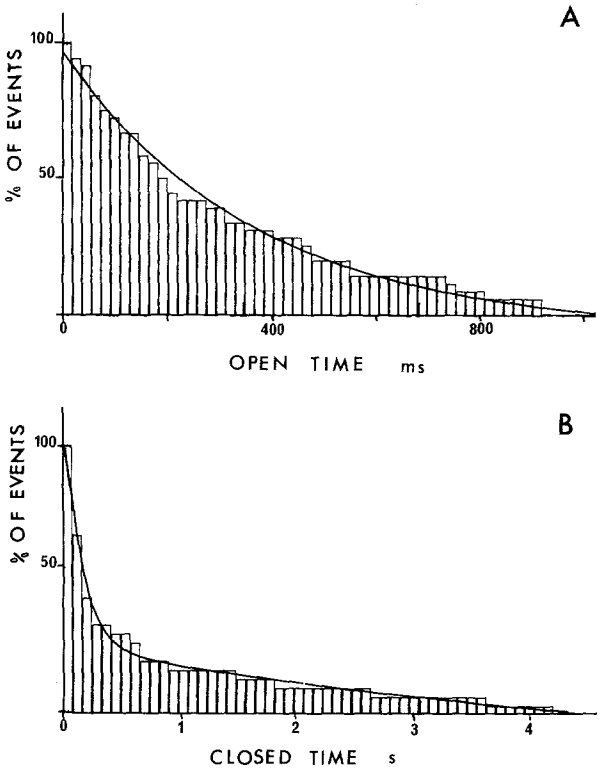


**Fig. 5.** Time histograms of open (*A*) and closed times (*B*). Open time was fitted with a single exponential with $\tau_{op}$ = 346 msec. Closed times were fitted with a double-exponential resulting in $\tau_{cfast}$ = 140 msec, and $\tau_{cslow}$ = 1.9 sec. Membrane potential −80 mV, 150 mM KCl-filled pipette

longer time in the open state which explains that $P_o$ increases with depolarization. The values for the short duration closed intervals are more scattered and it is difficult to assess for the voltage depen-

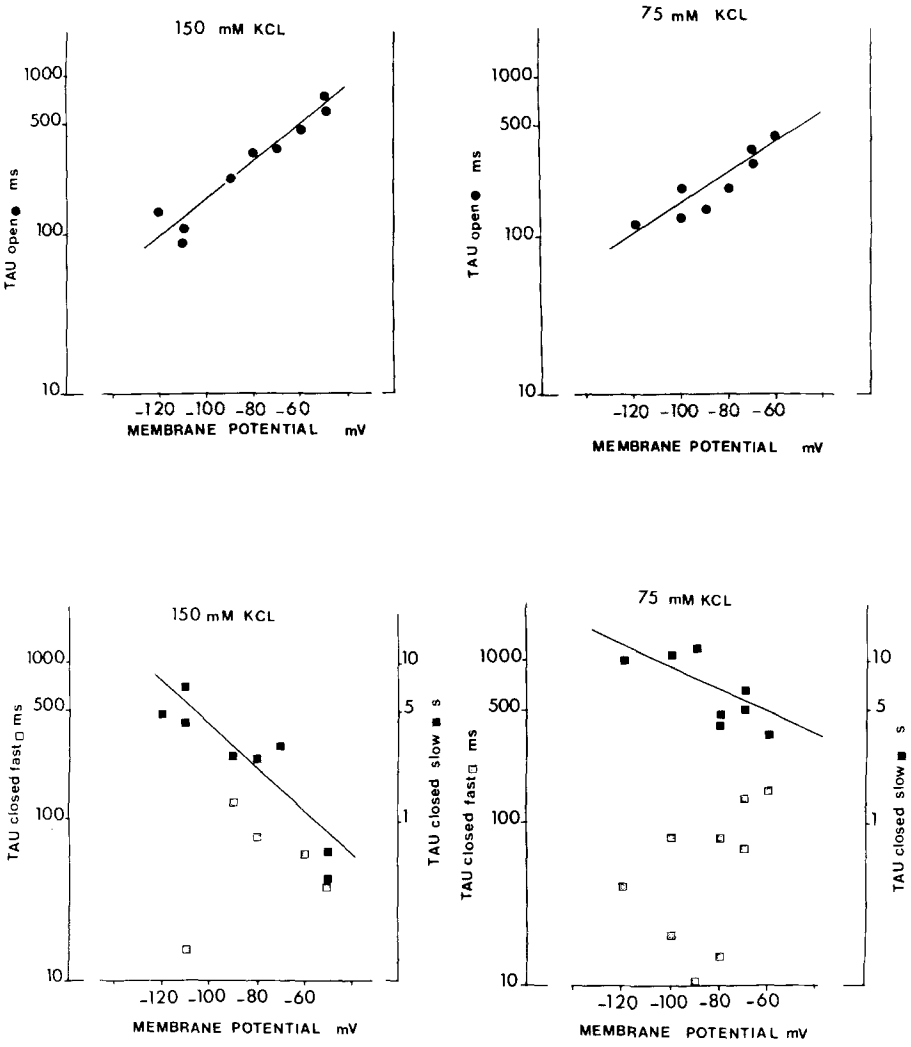


**Fig. 6.** Time constants for the open and closed states. Left panel: the time constants $\tau_{open}$, $\tau_{closed\ fast}$ and $\tau_{closed\ slow}$ are plotted as a function of voltage for results obtained with 150 mM KCl-filled pipettes. Right panel: time constants $\tau_{open}$, $\tau_{closed\ fast}$, $\tau_{closed\ slow}$ plotted as a function of voltage for 75 mM KCl-filled pipettes. Note that the points for $\tau_{closed\ fast}$ were not fitted due to a large scattering of the values

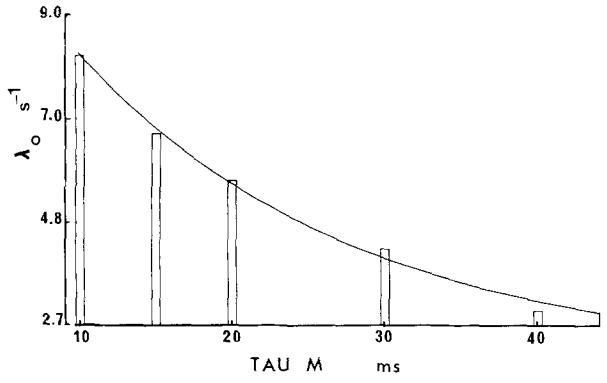


**Fig. 7.** Plot of $\lambda_o$ versus $\tau_m$. The approximative probability $\hat{P}_o$ was calculated with different $\tau_m$. For each $\tau_m$, the rate $\lambda_o$ is calculated and the plot $\lambda_o$ versus $\tau_m$ is fitted according to Eq. (2). The continuous line was obtained with the following values ($S^{-1}$): $K_1$: 54.9; $K_2$: 10.9; $K_3$: 2; $K_4$: 0.3. 75 mM KCl-filled pipette

dency of $\tau_{cfast}$. Comparison of the right panel (150 mM KCl) and the left panel (75 mM KCl) of Fig. 6 shows that $[K]_o$ does not affect $\tau_{open}$, the absolute values and the slopes of the straight lines being unchanged. The effect of $[K]_o$ on the kinetic of the channel seems to be mainly on $\tau_{cslow}$ and Fig. 6 shows that the slope of the relationship $\tau_{cslow}$-voltage changes in 75 mM KCl from 0.02 to 0.03. But more important is that the magnitude of $\tau_{cslow}$ is higher in low $[K]_o$ for depolarized voltages which results in a lower $P_o$.

## Rate Constants

The analysis of the open or closed time interval distributions was carried out using a half amplitude minimum time resolution approach. Within this framework, time intervals measured at half amplitude and smaller than a predetermined value were simply neglected (Methfessel & Boheim, 1982; Sachs et al., 1982; Moczydlowski & Latorre, 1983; Sakmann & Trube, 1984*a*,*b*).

We used a minimum time interval resolution of 10 msec in order to eliminate the very fast (<1 msec) kinetics that were otherwise present. We found, under these conditions, that the open and closed time interval distributions could be respec-

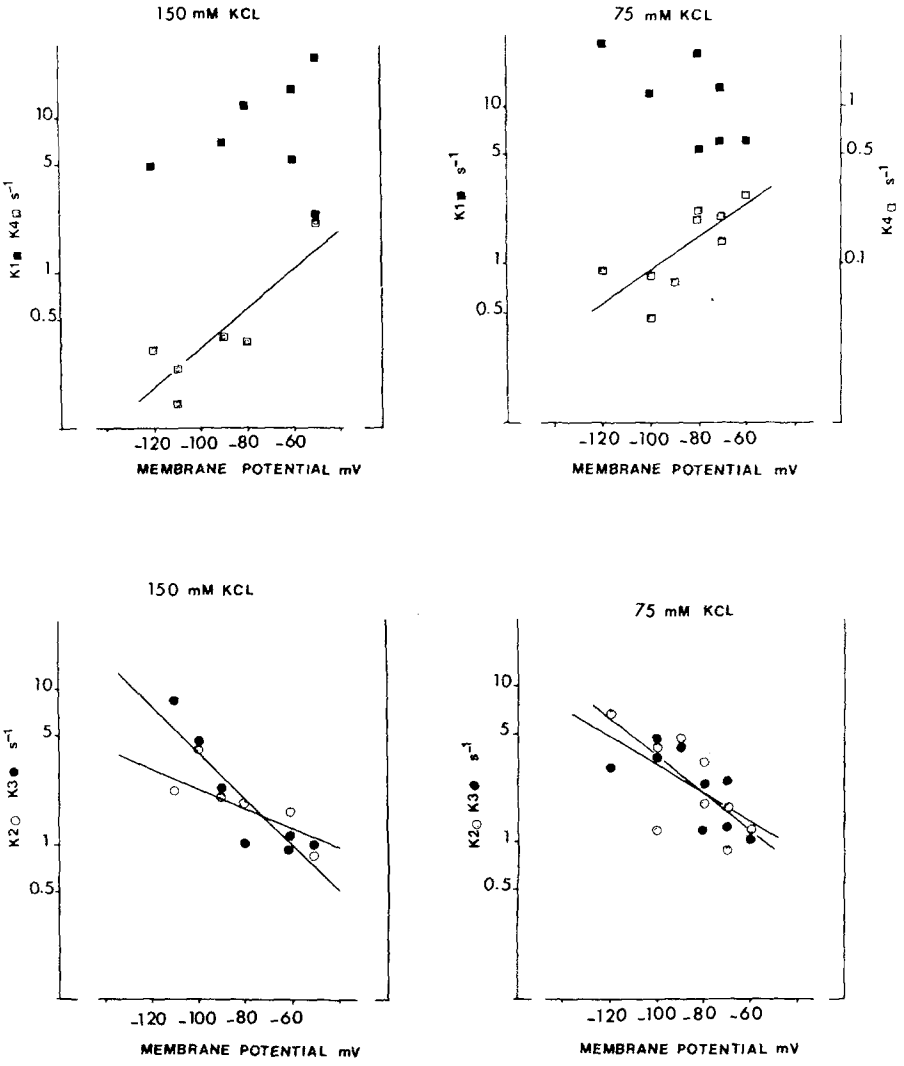


**Fig. 8.** Rate constants for the open and closed states. The rate constants $K_1$, $K_2$, $K_3$ and $K_4$ calculated according to a C-O-C model are plotted as a function of voltage for two different KCl concentrations. Left panel: rate constants for 150 mM KCl. $K_1$ and $K_4$ determine the closed-state duration (upper); $K_2$ and $K_3$ determine the open-state duration (lower). An $e$-fold change of $K_2$ is produced by a change of 71 mV of the membrane potential; 29 mV for $K_3$ and 33 mV for $K_4$. Right panel: rate constants for 75 mM KCl. $K_1$ and $K_4$ for the closed states (upper); $K_2$ and $K_3$ for the open state (lower). An $e$-fold change of the rate constants is produced by a change of 36 mV for $K_2$, 50 mV for $K_3$ and 41 mV for $K_4$. Note the different scale for $K_4$ in 75 mM KCl compared to 150 mM KCl. Due to the scattering of the points, the values for $K_1$ were not fitted

tively fitted by a single and by two exponential functions.

The rate constants of the transitions between the open and closed states were thus calculated for the simple C-O-C model from results obtained at 150 and 75 mM KCl.

An increase of the minimum time interval value $\tau_m$ from 10 to 40 msec did not change the number of exponentials involved in each case, but the resulting constant associated to each exponential was found to decrease as $\tau_m$ increases (*see* Fig. 7). In order to take this effect into account, we used the mathematical formalism proposed by Roux and Sauvé (1985) which predicts for the C-O-C model, namely:

$$C \underset{K_2}{\overset{K_1}{\rightleftarrows}} O \underset{K_4}{\overset{K_3}{\rightleftarrows}} C$$

that

$$\tilde{P}_o(\tau, \tau_m) = e^{\lambda_o \tau} \tag{1}$$

with

$$\lambda_o = -(K_2 e^{-K_1 \tau_m} + K_3 e^{-K_4 \tau_m}) \tag{2}$$

where $\tilde{P}_o(\tau, \tau_m)$ is the approximative probability of having a time interval greater than $\tau$. The values of $K_1$, $K_2$, $K_3$ and $K_4$ could thus be obtained by curve fitting to Eq. (2) to the various values of $\lambda_o$ obtained for different $\tau_m$ (*see* Fig. 7).

It follows, furthermore, that for $K_1$ and $K_4 \ll 100$ or $K_2 + K_3 \ll 100$ (*see* Roux & Sauvé, 1985) the effect of a value of $\tau_m$ fixed at 10 msec on both $P_o(\tau, \tau_m)$ or $P_c(\tau, \tau_m)$ ($c$ = closed) should be negligible. Under these conditions, the values of $K_1$, $K_2$, $K_3$ and $K_4$ could thus be calculated from

$$K_2 = A_1/\lambda_o$$
$$K_3 = A_2/\lambda_o$$
$$K_1 = -\lambda_1$$
$$K_4 = -\lambda_2$$

where $P_c(\tau) = A_1 e^{\lambda_1 \tau} + A_2 e^{\lambda_2 \tau}$.

Figure 8 shows that the rate constants $K_2$, $K_3$ and $K_4$ are voltage dependent; $K_2$ and $K_3$, which

determine the open lifetime, decreasing, and $K_4$, determining the lifetime of the long closed interval, increasing with depolarization.

Due to the scattering of the points no appreciable effect on the voltage dependency of $K_2$, $K_3$ and $K_4$ as well as on the magnitude of $K_2$ and $K_3$ could be attributed to $[K]_o$. A change of 33 mV and of 42 mV of the membrane potential is necessary for an $e$-fold change of $K_4$ in, respectively, 150 and 75 mM KCl. However, the magnitude of $K_4$ is decreased by a factor of about 4 in low $[K]_o$. In 150 mM KCl, $K_4$ values range from 0.17 $S^{-1}$ at −120 mV to 1.06 $S^{-1}$ at −60 mV and in 75 mM KCl from 0.056 to 0.24 $S^{-1}$ for the same voltages.

## Discussion

Patch-clamp experiments were carried out on myocytes of newborn rats with KCl-filled electrodes. We showed in this work that a particular type of single-channel event, referred to as type I, could be related to a $K^+$ channel with voltage and $[K]_o$ dependent open-closed kinetics.

The channel selectivity to $K^+$ was inferred from the zero-current potential shifts obtained at various KCl concentrations in the patch electrode. The measured values were fitted by the Goldman-Hodgkin-Katz equation with a $P_{Na}/P_K$ of 0.056. Hagiwara and Takahashi (1974) on starfish egg and Sakmann and Trube (1984*a,b*) on ventricular cell have also demonstrated that the inward rectifier channel is permeable to $Na^+$ ions but to a less extent ($P_{Na}/P_K < 0.03$ for starfish egg). However, despite their low permeability, $Na^+$ ions facilitate the K conductance for KCl concentrations lower than 50 mM. As proof, the unit conductance measured with a 20 mM KCl, NaCl-free medium-filled pipette is less than expected with KCl + NaCl medium. The facilitatory effect of $Na^+$ could be explained by considering that $Na^+$ binds on a $K^+$ site of the pore in which the single-file diffusion process is taking place (Hille & Schwarz, 1978).

Fukushima (1982) has postulated for the $Na^+$ facilitation of the K conductance of the anomalous K rectifier, that $Na^+$ ions may bind to the outer binding site of the pore and expel the $K^+$ ion binded on the innermost site in the internal space. The $Na^+$ binding is assumed to produce the closed state of the channel which is not the case in ventricular muscle where closed states are observable in Na-free solutions. It is nevertheless clear that $K^+$ is the main charge carrier and that a change in anion does affect neither the zero-current potential nor the single-channel conductance.

The $I/V$ relationship of this particular channel was linear at voltages more negative than the zero-current potential. However, the channel $I/V$ curve did not appear to be ohmic over the entire voltage range considered since no outward current jumps could clearly be detected especially with pipettes containing 150 mM KCl. It seems therefore that the channel conductance is substantially lower than 30 pS for potential values more positive than the equilibrium potential of $K^+$.

Our results also indicate that the open-channel probability does not increase at hyperpolarizing voltages as expected for an inward rectifying channel. On the contrary, we found that $P_o$ increased as more positive potentials were applied. In this regard, the results we obtain on the potential dependence of $P_o$ are similar to those reported by Benham and Bolton (1983) on the smooth muscle cells of rabbit jejunum, on $Ca^{2+}$-activated K channels seen on several preparations (*see*, for example, Moczydlowski & Latorre, 1983), on guinea pig heart cells (Sakmann & Trube, 1984*a,b*), and on rat's myocytes (Josephson & Brown, 1984). However, an inverse $P_o$ voltage relationship was observed for a $K^+$ inward rectifying channel on guinea-pig atrial cardioballs (Bechem et al., 1983).

It may be interesting to determine if the relationship between $P_o$ and $V_m$ shown in Fig. 4*A* results from a channel-blocking effect at hyperpolarized voltages as reported for some $K^+$ inward rectifier (Hagiwara et al., 1978; Ohmori, 1978; Fukushima, 1981, 1982). First, the degree of inactivation we observed with various pipette solutions cannot be due to an internal anion, since our experiments were carried out in the cell attached configuration where the internal medium remains basically unchanged. Secondly, a blocking effect of $Na^+$ (Fukushima, 1982) may be advanced but since we found that the value of $P_o$ decreases at hyperpolarizing voltages even if $Na^+$ was not present in the patch electrode, there should not be any major contribution of this ion to the voltage-dependent channel closure. In this regard, the $K^+$ channel described here does not correspond to the $K^+$ inward rectifier whose inactivation at hyperpolarizing voltages disappears in $Na^+$-free external solutions (Ohmori, 1978; Fukushima, 1981, 1982). One possible molecular mechanism that can explain the voltage dependence of $P_o$, would consist of a gating inactivation process as described by Sakmann and Trube (1984*a,b*). Within that framework, the channel is presumed to be fully activated at negative membrane potentials, so that the lower open-channel probability measured at hyperpolarized voltages is regarded as the resulting effect of a voltage-dependent inactivation process, a greater inactivation being related to a more negative transmembrane

voltage. The combined product of the activation and inactivation processes then appears as an increasing open-channel probability at depolarizing voltages, the channel being less inactivated at more positive membrane potentials.

The $[K]_o$ dependencies of $P_o$ may be explained by assuming that $K^+$ actually activates the channel. In fact, it has been already well established that permeant ions can influence the gating of both transmitter-activated or voltage-activated channels (Hagiwara & Yoshii, 1979; Marchais & Marty, 1979; Swenson & Armstrong, 1981; Junge, 1982). Our analysis of the C-O-C model indicates that the rate constant $K_4$ is mainly responsible for the effect of $K^+$ ions on the channel kinetics. It is thus likely that one or more $K^+$ interacted with the channel external site in such a way as to make the inactivation state of the channel less stable.

The kinetic analysis of the C-C-O model has also been carried out but the values obtained for the rate constants were found to be more scattered and less consistent. This does not, however, rule out the C-C-O model as one possible molecular mechanism in order to explain this particular channel kinetic behavior.

In conclusion, we have presented results on a K inward rectifying channel with $[K]_o$ and voltage-dependent kinetics. Similar results have been obtained on guinea-pig heart cells (Sakmann & Trube, 1984*a*,*b*) and HeLa cells (Sauvé, Roy & Payet, 1983), which seems to indicate that the molecular mechanism responsible for this inward rectifying current may be quite general and shared by several excitable or nonexcitable cells.

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