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SR47063, A Potent Channel Opener, Activates K_{ATP} and a Time-dependent Current Likely Due To Potassium Accumulation

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Abstract. (i) We studied the effects of a new cromakalim analogue, SR47063, in guinea-pig ventricular cells. The experiments were carried out in whole-cell patch clamp with internal and external solutions supposedly similar to the physiological ones. (ii) SR47063 reversibly activated a time-independent current reversing near the potassium equilibrium potential, and a time-dependent current reversing at a more positive potential. Both currents were blocked by application of glibenclamide. (iii)The time-independent and the time-dependent currents were activating for the same concentration of agonist in every cell, this concentration being very different from cell to cell. (iv) The amplitude of the time-dependent current was shown to depend directly neither on agonist concentration nor on potential, but rather on the amplitude of the current flowing during the prepulse before the test pulse. (v) We conclude that SR47063 is a potent K_{ATP} channel opener acting at concentrations lower than one micromolar, and that the time-dependent current is likely due to accumulation and depletion of potassium in restricted areas of the cells.

Key words: K_{ATP} — Potassium channel opener — Cromakalim — Patch clamp — Whole-cell recording — Accumulation

Introduction

Potassium channel openers, such as cromakalim (BRL34915), hyperpolarize various tissues via the activation of adenosine triphosphate (ATP)-sensitive potassium channels (Quast, 1992). In the heart, cromakalim and its analogues accelerate repolarization, and finally render the cells nonexcitable (Osterrieder, 1988; Sanguinetti et al., 1988; Escande et al., 1989; Faivre & Findlay, 1989; Findlay et al., 1989; Gautier, Bertrand & Guiraudou, 1991). This effect is specifically antagonized by the hypoglycaemiant molecule glibenclamide.

SR47063, [4-(2-cyanimino-1,2-dihydropyrid-1-yl)-2,2 dimethyl-6-nitrochromene], a structural analogue of cromakalim, is reported to decrease the duration of the action potential, this action being antagonized by glibenclamide (Gautier & Bertrand, 1991). The present study was designed to investigate the ionic current changes accounting for the effects of SR47063 observed on multicellular preparations. For this purpose, we recorded whole-cell currents in dissociated guinea-pig ventricular myocytes in the absence of inward current blockers. Experiments were carried out in whole-cell recording voltage clamp with an internal solution supposedly similar to the intracellular medium.

Our major finding is that, at room temperature, a submicromolar concentration of SR47063 induces a dramatic increase in time-independent current. The SR47063-activated current reverses at the potassium equilibrium potential E_K and is antagonized by glibenclamide. We conclude that SR47063 is a potent activator of the ATP-dependent potassium current I_{KATP}.

After a depolarizing test pulse, we recorded in the presence of SR47063, not only the time-independent current, but also a time-dependent current upon returning to a negative voltage. This current, whose properties appear similar to those of I_{KATP}, but which reverses at another equilibrium potential, is probably due to potassium accumulation in restricted spaces.

Materials and Methods

Animals were killed by cervical dislocation. Hearts were rapidly removed and cannulated on a Langendorff column, where they were

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perfused with the different solutions at 37°C. All solutions in the perfusion were bubbled with O_2. The dissection and a first 5 min rinsing step were done in calcium-free Tyrode (*see* Solutions). The heart was then perfused for 10–20 min with the enzyme solution, followed by 5 min in calcium-free Tyrode and 50 min in KB medium (Isenberg & Klöckner, 1982). The cells were kept at 4°C in KB and used only the day of preparation.

The effects of SR47063 were studied in whole-cell voltage clamp on isolated ventricular cardiocytes. The experiments were performed at 24–27°C in a 35 mm petri dish. The bath was continuously perfused by gravity at 0.7 ml/min.

SR47063 solution was prepared daily at 10 or 15 mM in ethanol by sonication at 35°C and diluted directly to the final concentration. Control experiments showed no effect of the solvent. In experiments using more than one concentration of SR47063, all solutions contained an equivalent amount of ethanol. Other experiments were carried out without ethanol in control. Glibenclamide was prepared similarly from a 2 mM solution in ethanol.

Solutions (Concentrations Given in Millimolar)

Calcium-free Tyrode

KCl, 5.4; NaCl, 137; $MgCl_2$, 1.05; glucose, 11.5; pyruvic acid, 5; thiamine, 0.1; HEPES, 10. pH = 7.2 (with NaOH).

Enzyme Solution

Twenty milligrams of collagenase type C0130 and 16 mg pronase XIV diluted a few minutes before the dissociation in 100 ml calcium-free Tyrode.

KB Medium

K-glutamate, 70; KCl, 25; KH_2PO_4, 10; $MgCl_2$, 5; oxalic acid, 10; taurine, 10; glucose, 11; pyruvic acid, 2; β-hydroxybutyric acid, 2; ATP, 2; creatine phosphate, 2; HEPES, 10. pH = 7.2 (with KOH)

Normal Tyrode

$CaCl_2$, 1.8 added to the calcium-free Tyrode.

High Potassium Solution

KCl, 26.4; NaCl, 116; $MgCl_2$, 1.05; $CaCl_2$, 1.8; glucose, 10; pyruvic acid, 5; thiamine, 0.1; HEPES, 10. pH = 7.2 (with NaOH).

Pipette Filling Solution

KCl, 20; K aspartate, 130; KH_2PO_4, 1; $MgCl_2$, 1; K-EGTA, 5; HEPES, 10. pH = 7.3 (with KOH).

Source of Products

Unless otherwise stated, chemicals were purchased from Sigma (St. Louis, MO). SR47063 was synthesized by Sanofi Recherche (Montpellier, France).

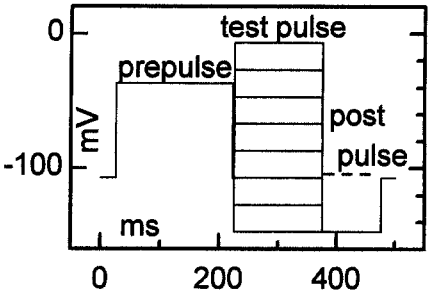

Fig. 1. Stimulation protocol. Standard stimulation applied to the preparation. Voltage jumps were applied continuously every 60 sec starting from the rupture of the patch. After the test pulse (from −7 to −147 mV by increments of −20 mV), the postpulse potential was either −147 mV or the resting potential (−107 mV). An interpulse of 3 msec to −107 mV between the prepulse and the test pulse was used to monitor changes in the series resistance. Experiments in which this resistance varied were discarded.

Electrophysiological Experiments

Tip Electrode Potential

The pipette solution was designed to resemble the intracellular medium. The aspartate anion is much less mobile than chloride so the pipette tip will be more permeable to Cl^- ion and behave partly as a chloride electrode. The zero current potential of the pipette in the bath is then negative. The liquid junction potential of −17 mV was computed (*see* Appendix A). When the patch is broken, we consider that after equilibration the cations and the anions are at equilibrium in the absence of current. The tip potential is then zero. The junction potential is compensated in the bath, so the pipette potential is actually −17 mV. In whole-cell voltage clamp, for a command potential of 0 mV, the inside of the cell is actually clamped to −17 mV. The voltages given below are compensated to their effective value.

Standard Stimulation Protocol

From a holding potential of −107 mV, the standard stimulation protocol consisted of an initial prepulse of −37 mV to partially inactivate the inward currents, followed by a test pulse from −147 to −7 mV by steps of 20 mV. After this pulse, the postpulse potential was either −107 or −147 mV (*see* Fig. 1). The stimulations were applied every 5 sec. A complete protocol (8 pulses, that is 40 sec) was applied every minute.

Materials

Current signals (RK300 Bio-Logic Amplifier, Bio-Logic, CLAIX, France) were filtrated with a 3 kHz 5-pole Bessel filter. No series resistance compensation was applied. Stimulation and acquisition of currents were performed (BioClamp software, Bio-Logic, CLAIX, France), through an I/O board (Labmaster, Scientific Solutions) at a sampling rate of either 1 or 2 kHz. The current traces were plotted with a dotted line indicating the zero current level.

Data Analysis

Steady-state currents were measured on-line by averaging samples over 3 msec, which provided smoother data than instantaneous measure-

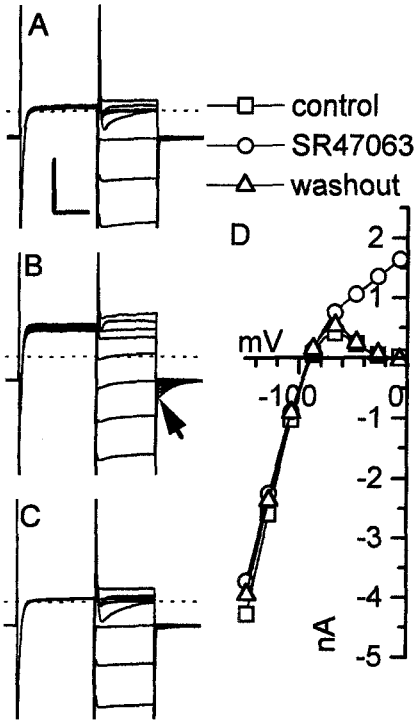

Fig. 2. Effect of 0.3 μM SR47063. (*A, B, C*) Currents recorded in control (*A*), after application of 0.3 μM SR47063 (*B*) and after washout (*C*). Scale bars: 100 msec 2 nA. Notice in *A* and *C* the presence of the calcium inward current during the test pulse, maximum at the most positive potential, −7 mV. In the presence of SR47063 (*B*), only a slight inward deflection is observed, at −27 mV. On return to −107 mV, the current is time independent in Tyrode (*A* and *C*), whereas a time-dependent "tail" is observed in the presence of SR47063 (*B*), as indicated by the arrow. (*D*) Current-voltage relation measured at the end of the pulse.

ments. Every computed current-voltage relation was stored as an ASCII file. A BASIC program gathered these files into a worksheet (Microsoft Excel).

Results

Effect of SR47063 on Membrane Current

Concentrations from 0.3 to 3 μM SR47063 altered the electrical properties of cardiac myocytes. We observed, however, a large disparity of results from cell to cell at a given concentration, which we discuss below.

A typical response of a ventricular myocyte to the standard stimulation is illustrated in Fig. 2*A*. The current during the prepulse was mainly the sodium one, declining to zero at steady-state. The current during the test pulse consisted of the inward rectifier for negative potentials, and a calcium current component for the most positive depolarizations. In these conditions, the zero current potential was close to the reversal potassium potential, E_K. The interpulse reduced the capacitive transient on hyperpolarization. In many cells, the activation phase of the inward rectifier (Kurachi, 1985; Tourneur et al., 1987) was followed by its slow inactivation (Sakmann & Trube, 1984; Biermans, Vereecke & Carmeliet, 1987), which could be due partly to potassium depletion (Standen & Stanfield, 1979). Upon returning to the holding potential, the current was weakly time dependent after the capacitive transient.

After application of 300 nM SR47063, the currents changed as shown in Fig. 2*B*. The outward current recorded during the prepulse was markedly increased. The currents recorded for positive voltage steps were also much more outward in an apparently time-independent manner, whereas the currents were only slightly affected on hyperpolarization. The current recorded upon returning to the holding potential was time dependent (*see* arrow in Fig. 2*B*), and appeared as an inward tail current. Whatever its nature, we call it hereafter "tail" current for simplicity.

After washout, these effects were reversed within a few minutes as indicated in Fig. 2*C*. The current during the prepulse was quite similar to the control, the large outward currents for positive test pulses were suppressed and the tail current disappeared. The steady-state current was plotted as a function of the potential in Fig. 2*D*. One can observe a clear increase for potentials more positive than E_K, whereas the current was slightly and reversibly reduced in the presence of SR47063 for negative potentials.

The time-dependent "tail" current was present in the presence of SR47063, but not in control or after washout, as displayed in Fig. 3*A* after the most positive test pulse. The instantaneous current during the post-pulse was plotted in Fig. 3*B* as a function of the test pulse potential. It was close to zero for the most negative voltage jumps and linear with voltage for potentials positive to the potassium equilibrium potential. It shows no sigmoid shape as expected for a "classical" activation curve.

In every experiment, the effect of SR47063 was quickly and fully reversible. Upon hyperpolarization, we observed a partially reversible effect which was either an increase (*see* Figs. 4, 5) or a slight decrease in current (Fig. 2). This discrepancy is discussed below. The following experiments were carried out to determine the ionic nature of the steady-state current activated by this molecule.

Effect of Potassium Concentration on the Effect of SR47063

The ionic nature of this current was investigated in cells whose conductance was increased by SR47063 at potentials more negative than E_K.

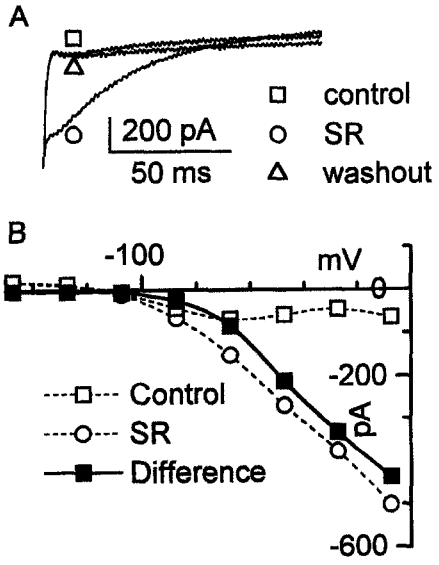

Fig. 3. Properties of the "tail" current (same experiment as Fig. 2). (*A*) Superposition of the time-dependent current recorded at −107 mV after a jump to −7 mV in control (squares), in the presence of SR47063 (circles) and after washout (triangles). The amplitude of the tail current was measured at the time indicated by the symbols for the current-voltage plot. (*B*) Current-voltage plot of the current measured in control (open squares), in the presence of SR47063 (open circles) and of the difference (filled squares, unbroken line). One observes a bell-shaped curve in control as expected from the activation properties of the inward rectifier. The difference measures the "tail" current due to SR47063. A similar diode-like response is observed when the tail current is recorded at −147 instead of −107 mV.

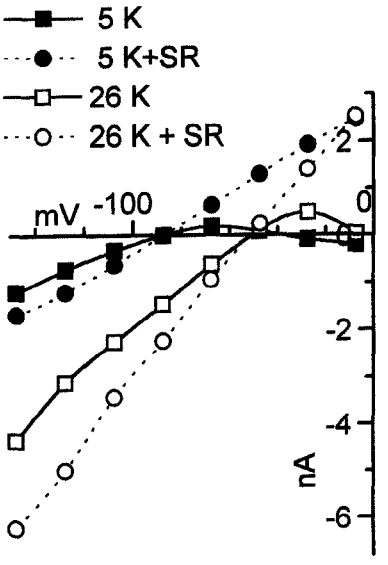

Fig. 4. Effect of potassium concentration. The protocol is the same as before, in particular the holding potential was kept the same in Tyrode and in high potassium. The experiment was performed in the following order: Tyrode (*5 K*), Tyrode + 1 μM SR47063 (*5K + SR*), Tyrode (washout, *not shown*), high potassium solution (*26 K*), high potassium + SR47063 (*26 K + SR*). Notice that SR47063 activates a larger current in high potassium solution than in Tyrode. The zero current potentials obtained by spline interpolation of these curves were: Tyrode: −87 mV; Tyrode + SR47063: −87 mV; high potassium: −50 mV; high potassium + SR47063: −50 mV. The shift in the zero current potential is +37 mV, the theoretical shift for a potassium electrode is +40 mV.

In the experiment illustrated in Fig. 4, the current-voltage relation in control Tyrode reflects the inward rectification and the negative slope conductance. In the presence of SR47063, the membrane does not rectify anymore. The two curves cross each other near the zero current potential for this cell. When returning to control, the curve was close to the initial one (*not shown*). Increasing the potassium concentration in the absence of potassium agonist shifted the zero current potential and increased the membrane conductance as expected for I_{K1}, with an enhanced inward rectification. In high potassium solution, in the presence of 1 μM SR47063, the currents are larger than in control and the cell does not rectify anymore. The current-voltage curve crosses the control one at the resting potential. The shift in the reversal potential of the agonist-activated current was similar to the shift of the zero current potential, close to the expected value for a potassium electrode. We conclude that SR47063 activated a potassium current.

Effect of Glibenclamide

The currents recorded under control conditions were plotted in Fig. 5*A*. We can observe the stable current after the sodium transient during the prepulse, and the development of an inward calcium current during depolarizing pulses for the most positive potentials. Upon hyperpolarization, the current was constant after the time-dependent activation of I_{K1}.

Application of 1 μM SR47063 (Fig. 5*B*) induced in this cell a large increase in the outward current for positive potentials and in the inward direction for negative voltage steps. We no longer observed the inward calcium current upon depolarization nor the activation of the inward rectifier upon hyperpolarization. The current during the test pulse appeared time dependent, except for −47 mV. After the jump from positive potentials to the resting potential, we observed a clear inward tail current, indicated by the arrow.

Application of 1 μM glibenclamide in the presence of SR47063 dramatically modified the previous effects, as indicated in Fig. 5*C*. The current during the prepulse returned close to zero, and the large outward currents for depolarizing pulses were almost suppressed. It looked as if the calcium current "recovered" slightly. On hyperpolarization, we observed a decrease in the steady-state inward current, and a recovery of time-dependent activation of I_{K1}. We also noticed that the tail current recorded after the test pulses was markedly reduced. These changes are reported on the steady-state current-voltage relation in Fig. 5*D*. The results from these experiments show that SR47063 is a potent and reversible activator of a potassium current that is blocked

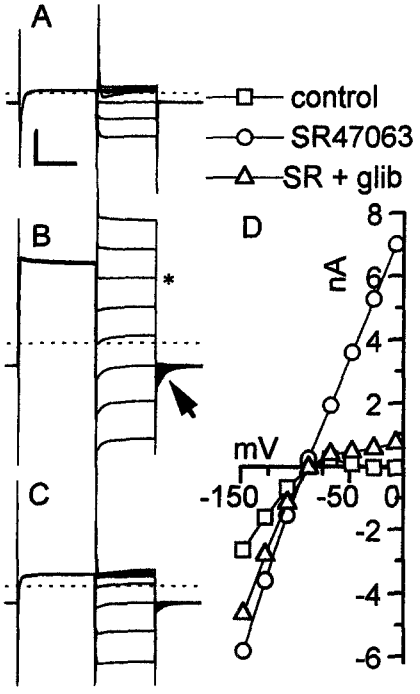

Fig. 5. Effect of glibenclamide after SR47063. (*A, B, C*) Currents recorded in control (*A*), in the presence of 1 μM SR47063 (*B*) and in the presence of SR47063 plus 1 μM glibenclamide (*C*). Scale bars: 2 nA, 100 msec. Note the large, outward time-dependent current flowing during the prepulse and the positive test pulses in *B*. The asterisk indicates the trace (–47 mV) at which this time-dependent current appears to reverse. The "tail" current (*see* arrow in *B*) is drastically reduced in the presence of glibenclamide. (*D*) Current-voltage relation measured at the end of the pulse.

by glibenclamide, a selective K_{ATP} blocker. We conclude that SR47063 is a K_{ATP} channel opener.

Overall Results

Out of 22 cells with concentrations of SR47063 ranging from 0.3 to 3 μM, we recorded an increase in outward current on depolarization in 19 cells; for potentials negative to E_K, we recorded either a reversible increase (n = 6) or decrease (n = 8) in current. In other experiments, no clear-cut effect, or a biphasic one (increase followed by a decrease) was observed. Out of the 19 cells in which the outward current increased on depolarization, 17 showed a clear tail current as described. The results obtained with SR47063 on 22 ventricular cells having stable currents are summarized in the Table.

In almost every experiment, we noticed that the effects of SR47063 on the steady-state current and on the "tail" current were simultaneously present or absent. It was then interesting to check the same cell's response to increasing concentrations of SR47063.

Effects of Varying SR47063 Concentration on the Steady-State and "Tail" Currents

Using the same protocol as in the previous experiments, we used here a more condensed presentation: the steady-state current at every potential was plotted (with a different symbol) as a function of time, at the constant rate of one protocol per minute. We can observe in Fig. 6*A* that the currents were not dramatically changed upon application of low concentrations of SR47063. The increase in the inward current at negative potentials may have been provoked by the application of the drug, although such an evolution might also be recorded in Tyrode. However, application of 3 μM SR47063 unambiguously induced an increase in outward current that was reversed rapidly upon returning to control. When the effect was maximum, the current-voltage relation was quite linear (*not shown*). This effect on outward current was not correlated with a similar increase in inward steady-state current on hyperpolarization.

In the same experiment, the tail current at –147 mV, measured as indicated in the inset, was plotted as a function of time in Fig. 6*B*. We can observe that the effects of SR47063 on both the steady-state current and on the "tail" current are simultaneous. This observation can be confirmed by plotting the effect as a percentage, for the steady-state current at –7 mV and the corresponding "tail" current (Fig. 7*A*). It is clear that the two curves may be superimposed.

Although the dose-response relationship was variable from cell to cell, we recorded a similar synchronous increase in the steady-state and the "tail" currents in another experiment with four concentrations, and in every other experiment (n = 12) checked with one concentration of SR47063. Never did we observe either an increase or a decrease in the steady-state current without the presence of a corresponding tail current. The "tail" current and the development of the outward current appear therefore linked.

The tail current may possibly reflect the time-dependent closing of K_{ATP} activated upon depolarization. When the current-voltage relation is linear, the proportion of open channels is probably the same at any potential, and the tail current is expected to be the same after different test potentials. We reported in Fig. 7*B* a combination of curves in Fig. 6*A* and *B*, i.e., the tail current as a function of the steady-state current during the preceding test pulse for all conditions.

For inward currents (negative on the x axis), the steady-state current varied slightly and no tail was recorded, so the corresponding points were close to the x axis. For outward currents (positive on the x axis), that is for test potentials evoking a response to the agonist, the tail current increased linearly as a function of the preceding current and depended neither on the agonist concentration nor on the potential.

Does the Tail Current Reflect Potassium Accumulation?

The relation between the preceding current and the tail amplitude suggests potassium accumulation in restricted

Table. Summary of experiments with SR47063

μM	Nb cells tested	Increase in outward K current[a]	Decrease in I_{KI}[b]	Increase in inward K current[b]	Activation of a tail current[c]
0.3	5	3	2		3
1	14	13	6	5	12
3	3	3		1	2
Total	22	19	8	6	17

Number of cells responding to SR47063 with one of the effects described in the text.
[a] Increase in outward current: for potentials more positive than E_K, SR47063 had either no effect or an effect of increase in outward current, and we report in the respective column the number of cells.
[b] Decrease in I_{KI}, increase in inward K current: For potentials negative to E_K, we recorded either a decrease in inward current, characterized as a decrease in the inward rectifier I_{KI}, or an increase in inward current, which may be the sum of different currents. Some cells showed no clear-cut effect on the inward current and are reported neither in increase nor in decrease.
[c] Activation of a tail current: this column refers to the cells responding with a clear and reversible "tail" current as defined above.

spaces. Such a phenomenon is well known on multicellular preparations (Attwell, Cohen & Eisner, 1979) and might also exist on single cells.

The existence of potassium accumulation can be tested with a two-step stimulation: a first step at a constant voltage followed by a second step to various potentials. This is achieved with our standard stimulation protocol by taking the prepulse as the first step and the test pulse as the second step. In this range of potentials, although the current appeared grossly time independent in control, a slight time dependence could be revealed at high gain (every trace has been shifted vertically to fit on the same plot), and is displayed in Fig. 8*A*. In control, the time-dependent current appears inward at −107 mV, outward at −67 mV and reverses close to −87 mV. In the presence of SR47063, the time-dependent current is much larger (*see* scale) and reverses positive to −67 mV, as if the current had become inward because of potassium accumulation during the prepulse. The measurement of the time-dependent current as indicated by the arrow was plotted as a function of the potential for the preceding currents (Fig. 8*B*). The zero current potential was close to −87 mV in control, and −53 mV in the presence of SR47063. Accumulation was then expected to shift the reversal potassium potential by 34 mV.

If this was the case, the instantaneous current following the depolarizing prepulse was expected to reverse at a different potential in control and in the presence of agonist. The currents recorded during the test pulse were reported in *C*. (Same traces as in *A*, but at a lower gain.) The dotted line indicates the zero current. It can be seen that the current trace at −87 mV was close to the zero line, in control as well as in SR47063. In the presence of this agonist, however, the initial time-dependent inward current (amplified in *A*) is observable.

The instantaneous current, measured at the time indicated by the arrow, was plotted as a function of potential in *D* in control and in the presence of SR47063. As it was clear from the original traces, these curves are close for negative potentials. They cross the voltage axis with a difference of 3 mV. One can then reject the idea that potassium may have accumulated by more than 0.5 mM in the vicinity of most of the channels during the prepulse, and that the potential at which the tail current reverses is due to global potassium accumulation.

Discussion

Since their discovery (Noma, 1983), the ATP-dependent potassium channels K_{ATP} were shown to be the target of a number of pharmacological agents (*for reviews:* Quast & Cook, 1989; Robertson & Steinberg, 1990; Longman & Hamilton, 1992; Quast, 1992; Weston & Edwards, 1992; Edwards & Weston, 1993). In the benzopyran family, like cromakalim and bimakalim, the new agonist SR47063 has drastic effects on the electrical and mechanical activity of cardiac preparations (Gautier & Bertrand, 1991). The present study was aimed at analyzing the mechanisms underlying these effects on single cells. We illustrated this analysis on a few experiments for clarity, although every assessment was confirmed in at least three experiments (except for the effect of increasing potassium concentration, two cells, and of four concentrations of agonist, two cells).

For potentials more positive than the potassium equilibrium potential E_K, we observed the development of an outward current upon the application of SR47063. In some experiments, SR47063 increased the potassium current at potentials more negative than E_K. The reversal potential of the current activated by SR47063 shifted along the voltage axis when the external potassium was raised from 5.4 to 26.4 mM as expected for a potassium current. This current was drastically decreased in the presence of 1 μM glibenclamide. In control experiments,

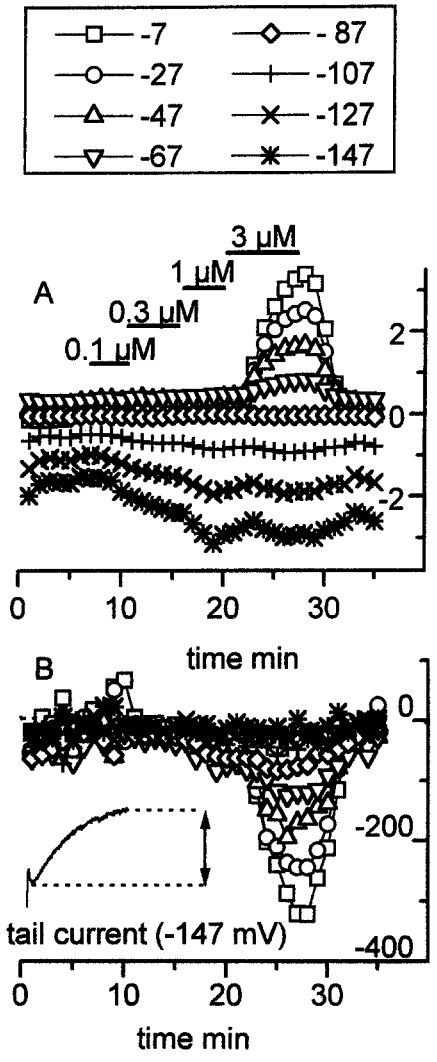

Fig. 6. Effect of increasing concentrations of SR47063 on the steady-state current and "tail" current. (*A*) Representation of the steady-state current as a function of time after the beginning of the experiment. The potentials are indicated in millivolts in the upper box. In this representation, in control, the points relative to outward currents (potentials positive to E_K) are superimposed and the negative slope conductance no longer appears. (*B*) Measurement of the corresponding "tail" current during the postpulse (at −147 mV) as indicated by the arrows (inset). Same time scale as in *A;* same symbols indicate the value of the preceding test potential.

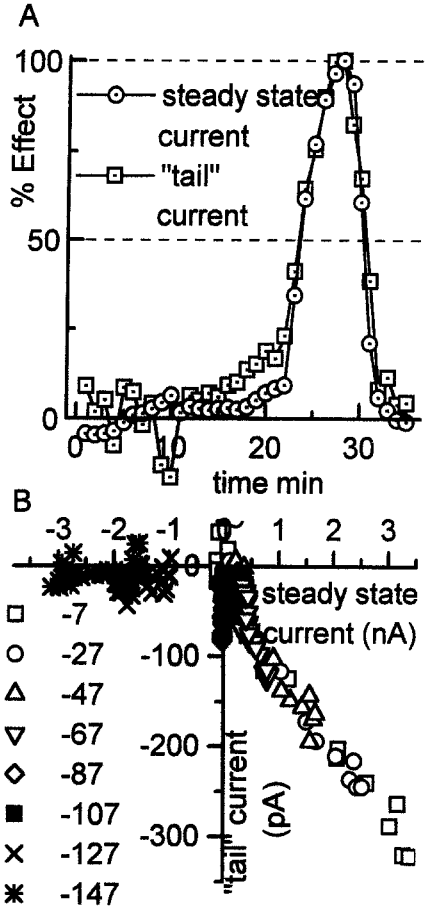

Fig. 7. (*A*) Amplitude of the steady-state current at the most positive voltage, −7 mV, and of the corresponding tail current, as a percentage of their relative maximum, plotted as a function of time. The steady-state current is measured as an absolute value, whereas the "tail" is measured as the difference between the initial and end-of-pulse values. Notice that although the scales of these currents are different, these two curves can be superimposed (same data as in Fig. 6). (*B*) Representation of the tail current as a function of the current during the preceding test pulse. When the current-voltage relation of a time-dependent current is linear, the proportion of activated channels is independent of the voltage and the tail current is the same after different values of potential. The current-voltage relation is quite linear at the maximum of SR47063 action (*not shown,* but *see* Fig. 6*A*). It is clear, however, that the tail current amplitude increases as the current during the test pulse increases.

application of glibenclamide (1 μM for 10 min) had no effect (*not shown*), confirming the observation of Escande et al. (1989). We conclude that SR47063 is a potent K_{ATP} channel opener. All experiments were carried out at room temperature (24–27°C). Since effects were observed at 0.3 μM, it appears that this molecule is far more potent than cromakalim (no effect of 30 μM at 22–24°C: Sanguinetti et al., 1988) or bimakalim (K_D = 6.8 μM, N_H = 1.7 at 24°C: Findlay et al., 1989).

The large variability of the effects of this molecule on K_{ATP} is remarkable, although we cannot exclude an effect of temperature within ±3°C. No data are available on the variability of the response of these channels to other openers, but their response to calcium (Findlay, 1988) and to ATP (Findlay & Faivre, 1991) were described to depend largely from cell to cell, and their response to glibenclamide to depend on the metabolic state (Findlay, 1993).

For potentials negative to E_K, the current was reversibly decreased by SR47063 in some experiments, whereas it was reversibly increased in others, apparently in similar conditions, without a clear concentration de-

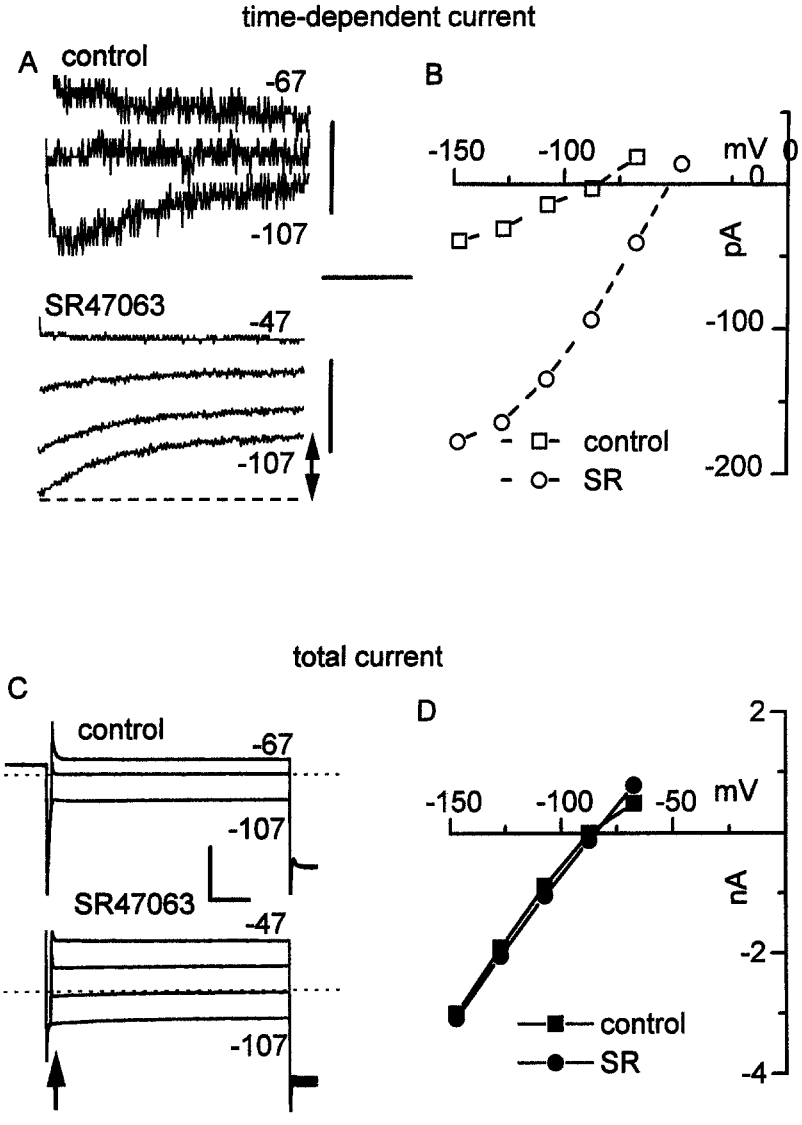

Fig. 8. Test of potassium accumulation. (*A*) Time-dependent current: The signal traces during the test pulse have been shifted vertically to fit close one to the other. Time scale, 100 msec. In control (from −67 to −107 mV, upper series of traces), the kinetics of the current appears time dependent at this magnification, and the current apparently reverses near −87 mV. Current scale: 50 pA. In the presence of SR47063 (from −47 to −107 mV, lower series of traces), the current is also time dependent and appears to reverse positive to −67 mV. Current scale: 250 pA. (*B*) Current-voltage relation of the time-dependent current, measured as the difference between the initial and the steady-state currents during the test pulse, as indicated by the arrow in *A*. (Open squares) control; (open circles) in the presence of SR47063. (*C*) Total current: Currents recorded during the test pulse, same traces as in *A*, but with the usual representation, showing also the end of the prepulse and the beginning of the postpulse. The zero current is the dotted line. Time scale: 50 msec, current scale for all traces, 2 nA. (*D*) Initial current-voltage relation of the total current (measured at the time indicated by the arrow) in control (filled squares) and in the presence of SR47063 (filled circles). The zero current potential in the presence of SR47063 is 3 mV positive to the zero current potential in control.

pendence for either effect. This might reflect an increase of K_{ATP} together with a blockade of the resting potassium conductance I_{K1}. The decrease in I_{K1} has been observed with other K channel openers: cromakalim (Conder & McCullough, 1987), pinacidil (Hiraoka & Fan, 1989; Nakayama et al., 1990; Tseng & Hoffmann, 1990), and nicorandil (Nakayama et al., 1991). Such similarities in the effects induced by different families of openers should not be surprising, since they may bind to the same receptor as in smooth muscle (Bray & Quast, 1992).

The linear current-voltage relation recorded in SR47063 in most experiments might suggest an artifact due to the series resistance when the membrane impedance became very low. However, even in experiments where the slope conductance was quite constant in the whole range of potentials, we could still increase it further by elevating the agonist concentration, the potassium concentration, or both. The current recorded was therefore not limited by the series resistance, so the actual membrane current-voltage relationship must then be linear. It is quite noticeable that although in every experiment the extra current activated by SR47063 was clearly rectifying in the outward direction, we never recorded an outward rectification of the whole-cell membrane current even in the presence of high concentrations of SR47063. Since the unitary conductance of the K_{ATP} channels is twice that of I_{K1}, and their numbers are equivalent (Noma & Shibasaki, 1985), it means that the open probability of K_{ATP} channels must always be less than half the open probability of I_{K1}.

What we have called "tail" current was observed after a jump from a potential at which the total current was outward to a potential at which it was inward, in experiments where the conductance upon hyperpolarization was increased as well as in others, where it was

decreased. The amplitude of the tail increases with the preceding potential. It activates for the same concentrations of agonist and simultaneously with the time-independent outward current on depolarization. It is also decreased as the outward current upon application of glibenclamide.

Does it reveal time-dependent properties of $I_{K_{ATP}}$? On rat ventricle, Zilberter et al. (1988) reported that the open probability of the channel decreases with the distance from the equilibrium potential. A tail current is then expected to decrease with the preceding positive potential, which is not the case in our preparation. In other studies, $I_{K_{ATP}}$ is described as time independent (Kakei, Noma & Shibasaki, 1985; Noma & Shibasaki, 1985; Qin, Takano & Noma, 1989) and voltage independent (Kakei et al., 1985).

In any case, when the current-voltage relation is linear for positive potentials, the activation variable is constant, and the tail must have the same amplitude after a jump from different potentials. Our results demonstrate that the amplitude depends only on the preceding current. The hypothesis of a time dependence of $I_{K_{ATP}}$ is thus inappropriate. Another explanation must therefore account for these results. We mentioned that the zero potential of the instantaneous current-voltage relation did not change by more than a few millivolts during the activation of the current. We cannot exclude the presence of another simultaneous, voltage-dependent ionic current, too small to significantly affect the zero current potential in conditions of high potassium conductance. If all currents were carried by potassium ions, no time-dependent current should be recorded at the reversal potential. Records in Fig. 8*A* show a large time-dependent component in the presence of agonist, even at −87 mV, which is close to the reversal potential (Fig. 8*B*). A current reversing at another potential was then flowing in addition to the inward rectifier and the ATP-dependent potassium current. The estimated value of its reversal potential is far from the potassium equilibrium potential. The tail current was suppressed with the blockade of K_{ATP} current by glibenclamide. Its amplitude increased with the amplitude of the preceding current, regardless of the potential and drug concentration, as a "memory" of the potassium flow during the preceding pulse. The vicinity of the membrane must then have changed during this flow. The reversal potential of the time-dependent current, then, cannot be interpreted with the known internal and external bulk solutions.

Such a change slightly affected the quasi-instantaneous current. We can exclude a contribution of the Na-K pump since it was fast and its direction was inverted at the most positive potentials. The simplest conclusion is that it did not concern the whole membrane, but rather some restricted spaces. The most likely change occurring during an outward potassium flow is extracellular accumulation of K^+ ions. According to this hypothesis, the reversal potassium concentration after the prepulse is given by the reversal potential, −53 mV in the described experiment, so the potassium concentration reaches a relatively high concentration, 36 mM. Simple computations given in the Appendix satisfy an estimate of 1.5 to 2.5% of the open channels being submitted to potassium accumulation. If we assume the number of K_{ATP} channels to be 2,000 to 3,000 per cell (Noma & Shibasaki, 1985), we find that the tail current involves only, at the most, one hundred channels. The possible effects of this local potassium accumulation due to $I_{K_{ATP}}$ in anoxia and ischemia have to be studied. The mechanism of potassium loss in these conditions is still questioned (Aksnes, 1992).

In some experiments, we observed a reversible suppression of the inward calcium and sodium currents. Effects of K channel openers on inward current have been reported in the literature (cromakalim: Sanguinetti et al., 1988; bimakalim: Sauviat et al., 1991). A study of the effects of potassium channel openers on inward currents, however, must be done carefully: potassium accumulation may induce time-dependent outward currents which superimpose on the inward currents, causing an apparent decrease. Such an artifact has been described on the effects of acetylcholine on the basal cardiac calcium current (Hartzell & Simmons, 1987).

In summary, we have shown that SR47063 is an activator of the ATP-dependent potassium conductance $I_{K_{ATP}}$. Effects could be recorded at room temperature at 300 nM, which is much lower than the concentrations used in studies on cromakalim and bimakalim. As for other potassium channel openers, SR47063 either increases or decreases the current recorded for potentials negative to E_K, regardless of the concentration. We consistently recorded the reversible presence of a time-dependent current simultaneous with the activation of $I_{K_{ATP}}$, which appears to depend only on the value of $I_{K_{ATP}}$ during the preceding pulse. This current is likely due to potassium accumulation in a restricted area of the cells, concerning a small fraction of the channels.

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References

Aksnes, G. 1992. Why do ischemic and hypoxic myocardium lose potassium? *J. Mol. Cell. Cardiol.* **24**:323–331

Attwell, D., Cohen, I., Eisner, D. 1979. Membrane potential and ion concentration stability conditions for a cell with a restricted extracellular space. *Proc. Roy. Soc. B* **206**:145–161

Biermans, G., Vereecke, J., Carmeliet, E. 1987. The mechanism of the inactivation of the inward rectifying K current during hyperpolarizing steps in guinea-pig ventricular myocytes. *Pfluegers Arch.* **410**:604–613

Bray, K.M., Quast, U. 1992. A specific binding site for K^+ channel openers in rat aorta. *J. Biol. Chem.* **267**:11689–11692

Conder, M.L., McCullough, J.R. 1987. Purported K channel opener, BRL 34915, blocks inwardly rectifying K current in isolated guinea-pig ventricular myocytes. *Biophys. J.* **51:**258*a* (*Abstr.*)

Edwards, G., Weston, A.H. 1993. The pharmacology of ATP-sensitive potassium channels. *Ann. Rev. Pharmacol. Toxicol.* **33:**597–637

Escande, D., Thuringer, D., Leguern, S., Courteix, J., Laville, M., Cavero, I. 1989. Potassium channel openers act through an activation of ATP-sensitive K^+ channels in guinea-pig cardiac myocytes. *Pfluegers Arch.* **414:**669–675

Faivre, J.F., Findlay, I. 1989. Effects of tolbutamide, glibenclamide and diazoxide upon action potentials recorded from rat ventricular muscle. *Biochim. Biophys. Acta* **984:**1–5

Findlay, I. 1988. Calcium-dependent inactivation of the ATP-sensitive K^+ channels of rat ventricular myocytes. *Biochim. Biophys. Acta* **943:**297–304

Findlay, I. 1993. Sulphonylurea drugs no longer inhibit ATP-sensitive K^+ channels during metabolic stress in cardiac muscle. *J. Pharmacol. Exp. Ther.* **266:**456–467

Findlay, I., Deroubaix, E., Guiraudou, P., Coraboeuf, E. 1989. Effects of activation of ATP-sensitive K^+ channels in mammalian ventricular myocytes. *Amer. J. Physiol.* **257:**H1551–H1559

Findlay, I., Faivre, J.F. 1991. ATP-sensitive K channels in heart muscle: spare channels. *FEBS Lett.* **279:**95–97

Gautier, P., Bertrand, J.P. 1991. Study of a new potassium channel opener, SR47063, on action potential and contraction of guinea-pig papillary muscle. *Fund. Clin. Pharmacol.* **5:**828

Gautier, P., Bertrand, J.P., Guiraudou, P. 1991. Effects of Sr 44866, a potassium channel opener, on action potentials of rabbit, guinea-pig and human heart fibers. *J. Cardiovasc. Pharmacol.* **17:**692–700

Hartzell, H.C., Simmons, M.A. 1987. Comparison of effects of acetylcholine on calcium and potassium currents in frog atrium and ventricle. *J. Physiol.* **389:**411–422

Hiraoka, M., Fan, Z. 1989. Activation of ATP-sensitive outward K^+ current by nicorandil (2-nicotinamidoethyl nitrate) in isolated ventricular myocytes. *J. Pharmacol. Exp. Ther.* **250:**278–285

Isenberg, G., Klöckner, U. 1982. Calcium tolerant ventricular myocytes prepared by preincubation in a KB medium. *Pfluegers Arch.* **395:**6–18

Kakei, M., Noma, A., Shibasaki, T. 1985. Properties of adenosine-triphosphate-regulated potassium channels in guinea-pig ventricular cells. *J. Physiol.* **363:**441–462

Kurachi, Y. 1985. Voltage-dependent activation of the inward-rectifier potassium channel in the ventricular cell membrane of guinea-pig heart. *J. Physiol.* **366:**365–385

Longman, S.D., Hamilton, T.C. 1992. Potassium channel activators drugs—mechanism of action, pharmacological properties, and therapeutic potential. *Med. Res. Rev.* **12:**73–148

Nakayama, K., Fan, Z., Marumo, F., Sawanobori, T., Hiraoka, M. 1991. Action of nicorandil on ATP-sensitive K^+ channel in guinea-pig ventricular myocytes. *Br. J. Pharmacol.* **103:**1641–1648

Nakayama, K., Fan, Z., Marumo, F., Hiraoka, M. 1990. Interrelation between pinacidil and intracellular ATP concentrations on activation of the ATP-sensitive K^+ current in guinea-pig ventricular myocytes. *Circ. Res.* **67:**1124–1133

Noma, A. 1983. ATP-regulated K^+ channels in cardiac muscle. *Nature* **305:**147–148

Noma, A., Shibasaki, T. 1985. Membrane current through adenosine-triphosphate-regulated potassium channels in guinea-pig ventricular cells. *J. Physiol.* **363:**463–480

Osterrieder, W. 1988. Modification of K^+ conductance of heart cell membrane by BRL34915. *NaunynSchmiedeberg's Arch. Pharmacol.* **337:**93–97

Pilsudski, R., Rougier, O., Tourneur, Y. 1990. Action of cromakalim on potassium membrane conductance in isolated heart myocytes of frog. *Br. J. Pharmacol.* **100:**581–587

Qin, D., Takano, M., Noma, A. 1989. Kinetics of ATP-sensitive K^+ channel revealed with oil-gate concentration jump method. *Amer. J. Physiol.* **257:**H1624–H1633

Quast, U. 1992. Potassium channel openers: Pharmacological and clinical aspects. *Fund. Clin. Pharmacol.* **6:**279–283

Quast, U., Cook, N.S. 1989. Moving together—K^+ channel openers and ATP-sensitive K^+ channels. *Trends Pharmacol. Sci.* **10:**431–435

Robertson, D.W., Steinberg, M.I. 1989. Potassium channel openers—New biological probes. *Annu. Rep. Med. Chem.* **24:**91–100

Sakmann, B., Trube, G. 1984. Voltage-dependent inactivation of inward-rectifying single-channel currents in the guinea-pig heart cell membrane. *J. Physiol.* **347:**659–683

Sanguinetti, M.C., Scott, A.L., Zingaro, G.J., Siegl, P.K.S. 1988. BRL34915 (cromakalim) activates ATP-sensitive K^+ current in cardiac muscle. *Proc. Natl. Acad. Sci. USA* **85:**8360–8364

Sauviat, M.P., Ecault, E., Faivre, J.F., Findlay, I. 1991. Activation of ATP-sensitive K channels by a K channel opener (SR44866) and the effect upon electrical and mechanical activity of frog skeletal muscle. *Pfluegers Arch.* **418:** 261–265

Standen, N.B., Stanfield, P.R. 1979. Potassium depletion and sodium block of potassium currents under hyperpolarization in frog sartorius muscle. *J. Physiol.* **294:**497–520

Sten-Knudsen, O. 1978. Passive transport processes. *In:* Membrane Transport in Biology. G. Giebisch, D. Toteson and H. Ussing, editors. pp. 5–113. Springer-Verlag, Berlin

Tourneur, Y., Mitra, R., Morad, M., Rougier, O. 1987. Activation properties of the inward-rectifying potassium channel on mammalian heart cells. *J. Membrane Biol.* **97:**127–135

Tseng, N.G., Hoffman, B.H. 1990. Actions of pinacidil on membrane currents in canine ventricular myocytes and their modulation by intracellular ATP and cAMP. *Pfluegers Arch.* **415:**414–424

Weston, A.H., Edwards, G. 1992. Recent progress in potassium channel opener pharmacology. *Biochem. Pharmacol.* **43:**47–54

Zilberter, Y., Burnashev, N., Portnov, V., Khodorov, B. 1988. Gating kinetics of ATP-sensitive single potassium channels in myocardial cells depend on electromotive force. *Pfluegers Arch.* **411:**584–589

Appendix A

Estimation of the Tip Potential

The ion exchanges around the pipette tip are too small to affect the bulk solutions in the bath and in the pipette. Because of different mobilities of the ions between the media, a potential develops which can be estimated (Sten-Knudsen, 1978, Eq. 195):

$$E = \frac{RT}{F} \frac{(Uo - Wo) - (Ui - Wi)}{(Uo + Wo) - (Ui + Wi)} \ln\left(\frac{(Uo + Wo)}{(Ui + Wi)}\right) \quad \text{(A1)}$$

where the subscripts o and i relate to the outward (bath) and inward (pipette) solutions, respectively, and U and W are the sum for all the ions in the solutions:

$$U = \sum_j u_j \cdot C_j \text{ with } u_j = \text{mobility cation } j \text{ and } C_j = \text{concentration cation}_j$$

$$W = \sum_j u_j \cdot C_j \text{ with } u_j = \text{mobility anion } j \text{ and } C_j = \text{concentration anion}_j$$

the mobilities are expressed in $m^2/sec/V$: Na^+: 5.1 10^{-8}; K^+: 7.4 10^{-8}; Cl^-: 5.1 10^{-8}. The mobility of aspartate is taken as that of tartrate, 1.32 10^{-8}. We assume that no tip potential exists when the cell is dialyzed. In these conditions, we find $E = -17.5$ mV, that is the reference potential is biased by this value. A tip potential of −17 mV has been experimentally measured in a preceding study, with glutamate instead of aspartate (Tourneur et al., 1987).

Appendix B

Estimation of the Fraction of Membrane Facing Potassium Accumulation

Overall Membrane Conductance in the Presence of SR47063

We shall assume in the following that one population of potassium channels is exposed to a uniform potassium accumulation in a restricted space. After a preceding voltage step to −37 mV, the reversal potential was estimated at −53 mV. We can estimate from the Nernst law that the potassium concentration is 43 mM. The maximum average cell conductance can be estimated as the slope of the steady-state current voltage relation. From −2,890 pA at −147 mV to 3,380 pA at −7 mV, the conductance is:

$$(3{,}380 + 2{,}890)/(147 - 7) = 44.5 \text{ nS}. \quad \text{(B1)}$$

Local Change in Conductance with Potassium Concentration

When potassium accumulates, the inward rectifier and K_{ATP} are affected. The conductance of IK_1 increases as the square root of the external potassium, whereas that of K_{ATP} increases as the power of 0.24 of external K (Kakei et al., 1985). The local membrane conductance varies between these two extremes. With an estimated potassium concentration of 43 mM, if the potassium conductance g_K changes as the square root of the external potassium, the local conductance is $\sqrt{43/5.4} = 2.8$ times the conductance at normal (5.4 mM) K. If it increases as the 0.24th power of potassium, the conductance is $(43/5.4)^{0.24} = 1.6$ times the conductance at normal K. The average local conductance is between 1.6 and 2.8 times the conductance at normal K.

"Tail" Current Amplitude

The initial tail current at −147 mV from a preceding potential of −37 mV can be estimated at a value of −170 pA from the current-voltage relation in Fig. 6*A* in this cell. We can estimate that the accumulation keeps $E_K = -53$ mV, the driving force can be calculated with $E_m = -147$ mV, so $E_m - E_K = -94$ mV. The conductance of this portion of membrane is then

$$\frac{I \text{ tail}}{(E_m - E_K)} = \frac{-170}{-94} = 1.8 \text{ nS} \quad \text{(B2)}$$

At normal potassium concentration, its conductance is between 1.6 and 2.8 times smaller, that is between 0.7 and 1.1 nS.

Fraction of the Channels Exposed to Accumulation

This conductance represents (0.7 nS) 1.5% to (1.1 nS) 2.5% of the 44.5 nS total cell conductance in normal K, suggesting that less than 3% of channels are exposed to this potassium accumulation.

Note Added in Proof

A recent study (Amsellem et al. 1994. Proceedings of the 13th International Congress of Electron Microscopy, **183**) indicates that 60% of the sarcolemmal membrane of guinea-pig ventricular cells is located in their transverse-axial tubular system. A partial access to a potassium conductance of this membrane may account for our results.