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Activation Properties of the Inward-Rectifying Potassium Channel on Mammalian Heart Cells

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Summary. The early phase of activation of the inward-rectifying potassium channel is studied on single cells from guinea-pig heart. The current is quasi-instantaneous when it is outward, but activates with time when it is inward. This relaxation is exponential and its time-constant decreases with hyperpolarization. The *I/V* curve reflects a strong inward rectification and has a negative slope conductance on depolarization. Similar results were recorded in the absence of sodium, calcium, chloride ions and in isotonic potassium. Cesium slows down the phase of activation, and eventually appears to block the channels by suppression of the activation. Barium, conversely, does not affect the activation, but promotes an 'inactivation' of this current, which blocks it. These results are independent on the cells' dissociation method. They suggest that this current is the inward rectifier, called I_{K1} on heart. Its activation curve suggests that the inward and outward currents are flowing through the same channels. The inward rectifier is time- and voltage-dependent on heart as on other tissues. The effects of cesium and barium are also similar. The importance of its negative slope conductance is discussed.

Key Words heart · ventricular cells · patch clamp · whole cell recording · inward rectification · potassium channels

Introduction

An inward-rectifying potassium conductance is present on many cellular types including the electric organ (Nakamura, Nakajima & Grundfest, 1965), nerve cell bodies (Kandel & Tauc, 1966), but has been mainly studied on skeletal muscle (Katz, 1949; Adrian & Freygang, 1962), and more recently on eggs (Hagiwara & Takahashi, 1974). Its main characteristics are identical on the different preparations (for review, *see* Stanfield et al., 1980). It is highly selective for potassium. On skeletal muscle (Leech & Stanfield, 1981) and eggs (Hagiwara et al., 1976), it rectifies instantly in the inward direction and activates with time on hyperpolarization so that it rectifies even more at steady state. Its kinetics when it is outward have not been studied extensively, but they appear much faster than when it is inward.

On heart, an inward-rectifying potassium conductance is also present in Purkinje fibers (Hall, Hutter & Noble, 1963), atrium (Rougier et al., 1968) and ventricle (Trautwein & McDonald, 1978). It has been called I_{K1} and is often referred to as 'instantaneous' current, or 'background' current. This current has been studied in single channel with the patch-clamp technique (Kameyama et al., 1983; Sakmann & Trube, 1984*a*,*b*; Payet et al., 1985). The existence of an outward current flowing through the same channels has been questioned (Sakmann & Trube, 1984*a*).

The inward-rectifying potassium conductance is blocked by barium and cesium on all these tissues (*see* Stanfield et al., 1980). For large hyperpolarizations, the inward current decreases with time because of potassium depletion and the effect of external sodium (Maughan, 1976; Ohmori, 1978; Standen & Stanfield, 1979), but also of a voltage-dependent inactivation (Sakmann & Trube, 1984*b*). Despite these similarities, the inward-rectifying potassium conductance on heart seems to display some specific properties (Sakmann & Trube, 1984*b*).

We used the patch-clamp technique in the whole cell recording configuration to study the activation of I_{K1} on single cells from guinea-pig heart. Our results show that this current activates with time on hyperpolarization and displays a negative slope conductance on depolarization. Cesium and barium induce a voltage- and time-dependent blockade as on other preparations. The inward and outward currents appear to flow through the same ionic channels.

These results extend the similarities between the inward-rectifying potassium channels from the different tissues.

Materials and Methods

Male guinea pigs (350 to 500 g) were injected intraperitoneally with heparin (4 units/g) (Elkins-Sinn, Inc., Cherry Hill, N.J.) 10 min before a lethal injection of pentobarbital (Nembutal, Abbot, Chicago, Ill.). The heart was quickly removed and cannulated under a Langendorff column, and perfused at 10 ml/min in Ca-free tyrode for 5 min.

The solution was changed to enzyme-containing solution (*see* Table), followed by rinsing in Ca-free tyrode for 5 min, and then 5 min in healing solution (*see* solutions below). At the end of the last perfusion, the heart was removed and kept in healing solution in the refrigerator. When needed, a chunk of tissue was cut and gently agitated in warm tyrode for dispersion. The cells adhered to the glass after a few minutes. The cells were usually used only one day.

The solution was warmed to 37°C and was continuously flowing in the experimental chamber on the microscope. Patch-clamp electrodes used capillary glass (Kimax 34502, Kimble, USA). The impedances in the bath were 2 to 5 MΩ and the seals ranged between 1 and 10 GΩ. The cells were stimulated at a constant rate between 0.05 and 0.2 Hz.

Computations were done on a Solar 16/40 (Bull-Sems, France).

Solutions

All the solutions were filtrated with a 0.2-μm pore filter before use. Concentrations are given in millimolar:

Tyrode

KCl, 5.4; NaCl, 137; $MgCl_2$, 2; NaH_2PO_4, 0.33; mannitol, 0.4; glucose, 10; pyruvic acid, 5; thiamine, 0.1; HEPES, 10; pH = 7.2 adjusted with NaOH. Eventually $CaCl_2$, 2 added. $CoCl_2$, 3 and diltiazem, 1 μM were added when necessary. In some experiments, the osmolarity has been adjusted to 300 mOsm with mannitol.

Na-free, Ca-free Solution

KCl, 5.4; $MgCl_2$, 1; mannitol, 280; $CdCl_2$, 5; mannitol, 0.4; glucose, 10; pyruvic acid, 5; thiamine, 0.1; HEPES, 10; pH = 7.3 adjusted with KOH.

Isotonic Potassium Sulfate Solution

K_2SO_4, 70; $MgSO_4$, 10; $CoCl_2$, 1; glucose, 80; pyruvic acid, 5; HEPES, 5; pH = 7.3 adjusted with NaOH.

Enzyme Solutions

Different methods provided a large number of good cells (Table). The enzymes and/or calcium chloride were added to Ca-free tyrode.

Table. Enzyme solutions[a]

Enzyme	Concentration (mg/ml)	Perfusion (mn)
Collagenase type II	2.4 + 36 μM $CaCl_2$	35
Protease type XIV	2.4	3
Collagenase type II + protease type XIV	2.4 + 0.24	20
Collagenase type 2139 + protease type XIV	2.4 + 0.24	20
Collagenase type I + protease type XIV	2.4 + 0.24	20

[a] The last solution appeared to provide the best yields and was used routinely. It could be modified to apply to different tissues (Mitra & Morad, 1985).

Healing Solution (Modified from Isenberg & Klöckner, 1982)

K-glutamate, 70; KCl, 25; KH_2PO_4, 10; $MgCl_2$, 5; oxalic acid, 10; taurine, 10; glucose, 11; pyruvic acid, 2; betahydroxybutyric acid, 2; ATP, 2; creatine phosphate, 2; penicillin G; 100 units/ml; streptomycin sulfate, 100 μg/ml; HEPES, 10; pH = 7.2 adjusted with KOH.

Intracellular-like Solution Filling the Patch-Clamp Micropipettes

K-glutamate, 115; NaH_2PO_4, 10; ATP, 2; creatine phosphate, 2; HEPES, 5; pH = 7.4 adjusted with KOH. In some experiments, 1 mM EGTA was added before pH buffering in order to prevent the cell contraction.

All chemicals came from Sigma (St. Louis, Mo.). ATP was vanadium-free. CsCl (5 mM) and $BaCl_2$ (0.5 mM) were eventually added to the control solution.

Results

Atrial and ventricular cells obtained by different methods had clear-cut striations and jagged ends. No difference in morphological or electrophysiological properties could be detected between the cells obtained by the five different dissociation methods.

In normal tyrode solution, the action potential ranged between 115 and 125 mV in amplitude and 200 to 500 msec in duration, measured either with a conventional microelectrode or a patch pipette in whole-cell clamp mode. The membrane potential was −75 to −85 mV when measured with a microelectrode, and −65 to −75 mV when measured with a patch pipette. This difference arises likely from a tip potential. The voltage difference between a microelectrode and a patch pipette, both free in the same bath solution, is shifted by 17 mV negative

(standard deviation 5 mV, 6 electrodes of each) when the bath is changed from tyrode to the internal solution. If the tip potential is the same in the cell and in the internal solution, then this voltage shift corresponds to the underestimation of the membrane potential in patch clamp. The following experiments have been performed with this technique and the potential of the cells was taken arbitrarily to −80 mV in the results. The recordings in one cell could last typically for 1 hr, and up to more than 3 hr with stable currents, which indicates that the internal solution was suitable for this preparation.

Tyrode Solution with Calcium Blockers

In order to study the potassium conductance, we suppressed the inward currents. The slow inward calcium current was blocked by the presence of cobalt and diltiazem, and the fast inward sodium current, which is poorly blocked by tetrodotoxin on this tissue, was inactivated by a depolarizing prepulse to −30 mV.

Figure 1(*A*) shows the typical protocol used: a 50-msec prepulse to −30 mV precedes a test pulse to different voltages. The fast inward current is quite inactivated at the end of the prepulse.

During the test pulse, the current is much larger for a similar voltage jump when it is inward than when it is outward, indicating a strong inward rectification. For potentials positive to the resting potential, the current appears time independent after the capacitive transient. For very positive potentials, increasing the depolarization decreases the outward current, which indicates the presence of a negative slope conductance. For potentials negative to the resting potential, after an initial jump, the current increases with time in the inward direction. This current activates with a single time constant, which depends on potential. The activation is faster for more negative potentials. The time constant of the current is plotted in Fig. 1(*B*) against voltage. The relation is roughly linear in a semi-logarithmic scale, in the milliseconds range.

The current measured at the end of the capacitive transient (open circles) and at steady state (filled circles) have been plotted on Fig. 1(*C*) (other cell) against potential. This plot reflects the nonlinear conductance of the membrane on depolarization, and has a negative slope for very positive potentials. Conversely, the curves appear steeper and linear on hyperpolarization, either for "instantaneous" values (broken line), or at steady state (full line).

For longer or more negative voltage steps, the

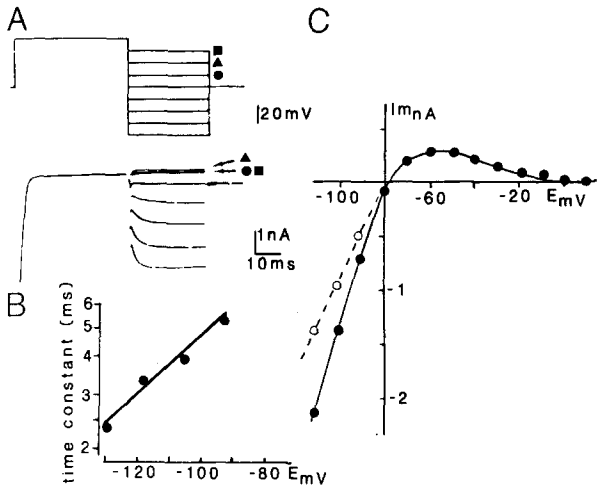

Fig. 1. Membrane currents in tyrode containing calcium channel blockers. The holding potential is the resting potential $E = -80$ mV. (*A*) General protocol: voltage test pulses are preceded by a prepulse (upper traces). The current records are superimposed (lower traces). The sodium inward current (peak value offscreen) is inactivated by a prepulse. Note the time-dependent current during the hyperpolarizing test pulses. Symbols indicate the presence of a negative slope conductance for positive test pulses. (*B*) Plot of the time constant of the time-dependent inward current against potential in a semi-logarithmic scale. Note that the time-constant decreases with increasing hyperpolarization (same experiment as in *A*). (*C*) Current-voltage relation for the quasi-instantaneous current (open circles) and the current measured at 25 msec (filled circles). Note the negative slope conductance in the depolarizing direction (other experiment)

inward current decreased with time (*not shown here*), as described by Sakmann and Trube (1984*b*). However, these conditions were not studied in detail, since it appeared difficult to distinguish between the development of the inactivation process and the potassium depletion from extracellular spaces.

Similar properties have been observed in atrial (average input resistance 100 MΩ) and ventricular cells (average input resistance 20 MΩ). We did not investigate further the initial quasi-instantaneous jump in the following results because of the time resolution of the method, but we focused on the time-dependent current.

The outward current decreases with voltage for potentials positive to the potassium equilibrium potential. This may either be due to the decrease of an outward potassium current, or to an inward current which has not been suppressed by cobalt and diltiazem. In the following experiment, we used another inward current blocker, cadmium, and we suppressed calcium and sodium from the external medium.

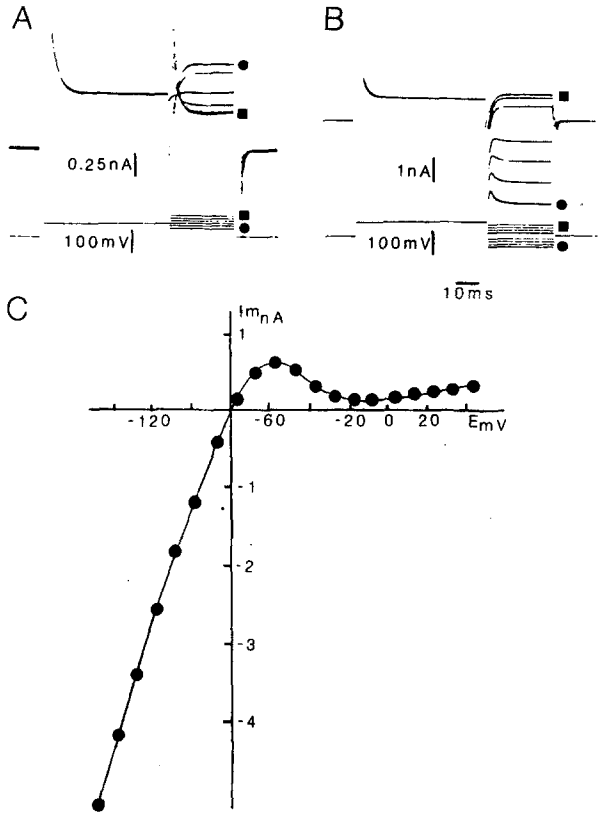

Fig. 2. Membrane currents recorded in Na- and Ca-free solution with 5 mM Cd. The holding potential is −80mV. Same protocol as Fig. 1. (*A*) Depolarizing voltage pulses in the region of negative slope conductance (*see* symbols). Note the absence of inward current during the prepulse. (*B*) Hyperpolarizing voltage pulses, showing the time-dependent current as in tyrode. (*C*) Current-voltage relation for the current recorded at 25 msec showing a clear negative slope conductance in the depolarizing direction. The positive slope conductance (0.3 GΩ) for very positive potentials is likely due to a leak

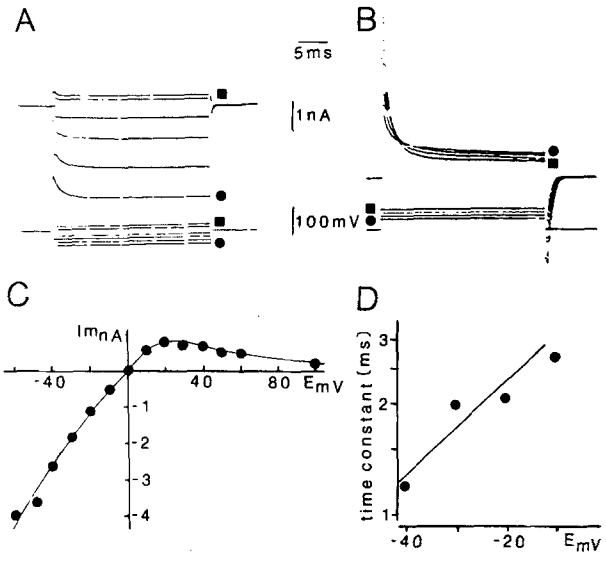

Fig. 3. Membrane currents in isotonic potassium sulfate solution. The holding potential is the resting potential, $E = 0$ mV. (*A*) Hyperpolarizing voltage pulses showing a time-dependent current similar to the current in normal K. (*B*) Depolarizing voltage pulses in the region of the negative slope conductance (*see* symbols). (*C*) Current-voltage relation for the current measured at 25 msec. Note the negative slope conductance in the depolarizing direction. (*D*) Plot of the time constant of the time-dependent inward current against potential in a semi-logarithmic scale

Sodium-free and Calcium-free Solution (+5 mM Cd)

We used a high concentration of cadmium which blocks all the calcium currents (Noble, 1984) and also the fast sodium current (DiFrancesco et al., 1985).

When normal tyrode is replaced by sodium-free, calcium-free, cadmium-containing solution, the cell depolarizes by less than 5 mV. After equilibration, the same voltage protocol is applied. In Fig. 2(*A*), the currents obtained for positive voltage pulses appear time independent after the capacitive transient, and indicate the presence of a negative slope conductance (*see* symbols). In Fig. 2(*B*), the currents for negative voltage steps appear much larger, and time dependent as earlier. Note the total absence of inward current during the prepulse, as expected.

The steady-state current-voltage curve is plotted in Fig. 2(*C*). The curve in Na- and Ca-free medium appears similar to the previous one; it displays a strong rectification. The outward current has a negative slope conductance for potentials positive to 25 mV from rest. These results rule out the possible effect of any remaining inward sodium or calcium current.

The voltage and time dependence of the current is then similar in the absence of sodium and calcium, and in the presence of cadmium.

Isotonic Potassium Sulfate Solution

The following experiment has been carried out in an external medium close to the solution used on skeletal muscle by Leech and Stanfield (1981) to study the kinetics of the inward-rectifying potassium channel.

In this solution, the resting potential is zero, and no prepulse is used. Figure 3(*A*) shows the currents recorded for various voltage steps. The membrane rectifies strongly, and the current is time dependent on hyperpolarization, but not on depolarization. The traces in Fig. 3(*B*) show the presence

of a clear negative slope conductance. The plot of the steady-state current against potential (Fig. 3*C*) shows the rectification of the cell. The outward current has a negative slope conductance for potentials positive to 25 mV from rest as in normal potassium. As in the previously described experiments, the time course of the current appears monoexponential. The time constant decreases with hyperpolarization, as shown in Fig. 3(*D*).

The ionic nature of the time-dependent current on hyperpolarization may be questioned. This current is still present in normal potassium concentration, when the chloride concentration is drastically reduced, as shown in Na- and Ca-free medium. The latter experiment in 2 mM chloride medium excludes the possibility of an inward time-dependent chloride current, as described on a molluscan neurone (Chesnoy-Marchais, 1983).

This time-dependent current resembles the time-dependent inward-rectifying potassium current on eggs and skeletal muscle (Hagiwara, Miyazaki & Rosenthal, 1976; Leech & Stanfield, 1981). In order to confirm the hypothesis of a similar activation on heart, we used the blockers of the inward rectifier, cesium and barium.

Effects of Cesium (5 mM) and Barium (0.5 mM)

The following experiments on the effects of cesium and barium have been carried out in the presence of the calcium blockers.

With the same protocol as earlier, the current recorded for one potential is displayed in Fig. 4(*A*), filled circle. When cesium is progressively added to the solution, the time-dependent current is slowed down. The time-dependent and steady-state currents are dramatically reduced (*see* arrow), and completely suppressed after equilibration (open circle). Figure 4(*B*) represents the current measured at the end of the test pulse in control and in cesium as a function of potential. It can be noticed that the currents have been much reduced in the inward direction, whereas the currents in the outward direction are little affected. For negative voltage steps, a negative slope conductance can be observed, which may indicate that the block is voltage dependent. The inset represents the percentage of block as a function of potential. It shows a monotonous decrease which can be fitted by a sigmoid curve.

The effects of barium were tested on another cell (Fig. 4*C*). The current in control (filled circle) is reduced by addition of barium (*see* arrow). In this case, the activation process is not clearly affected, but barium promotes a block looking like an inactivation. The time-dependent and steady-state currents are here completely suppressed after equilibration (open circle). The current-voltage curves in control and in barium are shown in Fig. 4*D*. The currents in the inward and in the outward direction have been suppressed by barium.

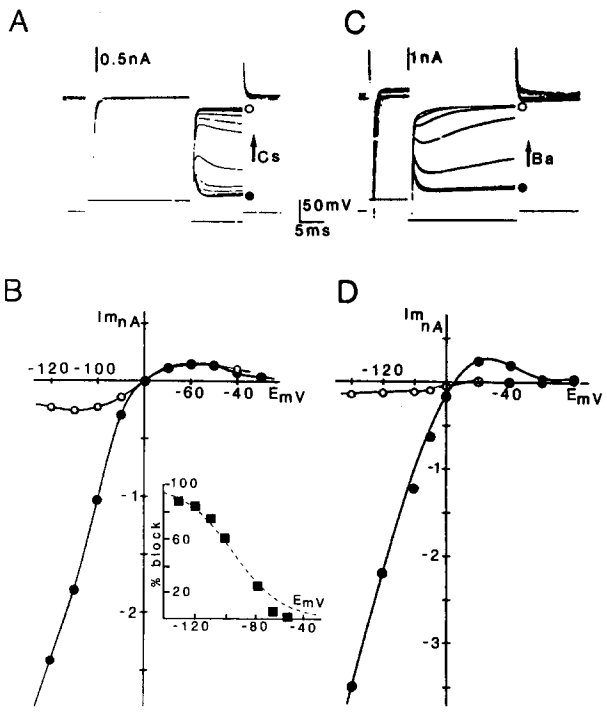

Fig. 4. Blocking action of cesium and barium. The holding potential is the resting potential $E = -80$ mV. The sodium inward current is inactivated by a prepulse to -30 mV. (*A*) Progressive blockage by Cs of the current during a hyperpolarizing voltage pulse: cesium delays the onset of the current. (*B*) Current voltage relation for the current measured at 25 msec, in control (filled circles) and in 5 mM Cs (open circles). Note the voltage dependence of the inhibition, which is plotted in inset as the ratio of the current in Cs to the current in control. (*C*) Progressive blockage by Ba of the current during a hyperpolarizing voltage pulse. Barium promotes an 'inactivation'-like process of the current. (*D*) Current-voltage relation for the current measured at 25 msec in control (filled circles) and in 0.5 mM Ba. Note that Ba blocks the current in the outward as well as in the inward direction

These effects of cesium and barium can be totally reversed.

The time-dependent current which is present in the absence of sodium, calcium, chloride ions, and which is reversibly blocked by barium and cesium can then be identified as the inward-rectifying potassium current. This current deactivates instantly or very quickly on depolarization, but activates with time on hyperpolarization. Its block by cesium is voltage dependent.

Barium, 0.5 mM, blocks the inward and the outward currents, and cesium blocks the inward cur-

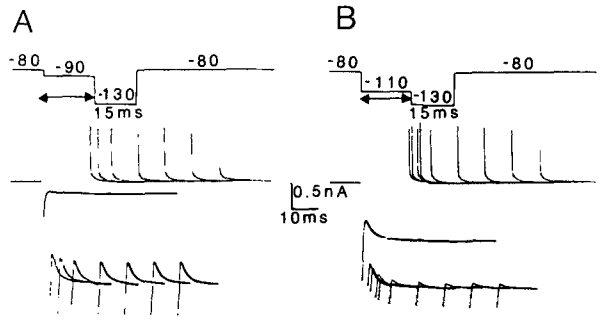

Fig. 5. Study of the time-dependent activation of the current by a two-step protocol. Note that the amplitude of the initial current during the second step is proportional to the current during the first step, for two different prepulses (*A*, *B*)

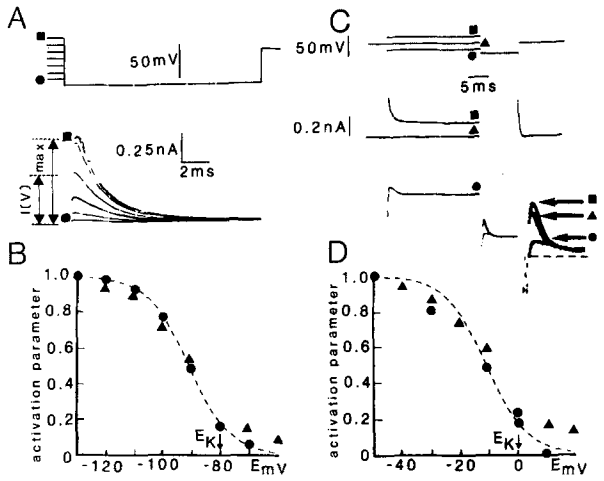

Fig. 6. Estimation of the activation curve in normal K (*A*,*B*) and in isotonic K (*C*,*D*). The holding potential is the resting potential ($E = -80$ mV in normal K and $E = 0$ mV in isotonic K). (*A*) Upper traces: two pulses voltage protocol. Lower traces: superimposition of the initial currents recorded during the test pulse. The other current traces were offscreen. The actual current values used for the computations were obtained by extrapolation to $t = 0$ of the traces. (*B*) Values of the activation parameter (circles) obtained from the preceding experiment, as described in the text, against potential. The dotted line was obtained by fitting by the following equation: $y = 1/(1 + \exp((E - E_1)/E_2))$, with $E_1 = -91$ mV and $E_2 = 7.2$ mV. Relative membrane conductance (triangles), defined as the ratio of the current to the driving force. In this solution, the membrane current is assumed to be identical to the inward-rectifying potassium current, and the driving force is therefore the difference between the membrane potential and the zero-current potential. (*C*) Same experiment as in (*A*), but in isotonic K sulfate solution. The current traces during the test pulse are enlarged in the inset. The dotted line represents the steady current at the end of the test pulse. (*D*) Activation parameter, computed as earlier, against potential (circles) together with the relative membrane conductance (triangles). The dotted line is not computed to fit these data, but is replotted from (*B*)

rent, but not the outward current. This might be due to the existence of different types of channels. The existence of an outward current flowing through the inward rectifier has also been questioned in single-channel recording (Sakmann & Trube, 1984*a*). In order to answer this question, the activation curve of the time-dependent current was studied in sodium- and calcium-free solution with 5 mM cadmium.

Activation Curve in Sodium- and Calcium-free Solutions

We used a double-step protocol to measure the instantaneous current in a way close to the measurements of tail currents. In the experiment described in Fig. 5, a 15-msec test pulse to -130 mV was preceded by a prepulse to -90 mV (*A*) or -110 mV (*B*), for various durations. We can observe that the envelope of the initial values of the current during the test pulse follows the time course of the current during the prepulse. The initial value of the current can then be considered as an indication of the degree of activation of the conductance.

In a second series of experiments, the potential was stepped to various voltages for a constant duration (Fig. 6*A*) before the test pulse. At any potential, the current had reached a steady value during the prepulse. The currents were recorded at high gain during the test pulse. From the initial amplitude, it is possible to compute the activation parameter, defined as: activation parameter $= (I(V) - I_{max})/I_{max}$, where $I(V)$ is the initial current measured after a prepulse to potential V, and I_{max} is the maximum value of this current (*see* Fig. 6).

This has been done on Fig. 6(*B*) (circles). With this method, the values of the activation parameter are 1 at the test pulse potential and 0 for the most positive value of potential. The plot can be fitted by the sigmoid dotted line. This result clearly indicates that the conductance is not quite deactivated when the potential is positive to rest, where the current flows outward. It suggests strongly that at least part of the outward time-independent current and the inward time-dependent current flow through the same channels.

By comparison, we superimposed on this curve the relative conductance obtained by dividing the total current (instantaneous + time-dependent current) by the driving force (triangles). The symbols are very close to the same curve, which tends to indicate that the time-dependent activation of the current and the membrane conductance are linked properties.

Similar experiments have been carried out in isotonic potassium solution. The protocol is shown in Fig. 6(*C*). The activation parameter has been esti-

mated as previously described (circles) and the relative conductance by dividing the total current by the driving force (triangles). On this plot, the dotted line re-plotted from (*B*) and shifted by 80 mV, recalls the results in normal potassium.

We can observe that the activation parameter is reasonably fitted by the curve obtained in normal potassium solution, after a shift along the voltage axis like the potassium equilibrium potential. In both solutions, the rectification of the membrane appears then due at least partly to kinetic properties of the channels.

Discussion

We have described in this paper electrophysiological properties of ventricular cells, studied with the patch-clamp technique in whole cell current mode. Similar results have been recorded with different cell dissociation methods, including pure collagenase (Isenberg & Klöckner, 1982) and pure pronase (Bustamante, Watanabe & McDonald, 1982). Any effect due to the proteolytic enzymes is then unlikely. The same properties could be recorded on atrial cells. They have also been observed on frog atrium and ventricle (Pilsudski & Tourneur, *unpublished*). This current is recorded in the presence of ATP inside the cell, so it cannot be the ATP-dependent current (Noma, 1983).

After suppression of the inward conductances, the current appears "time independent" when it is outward, and time dependent after a quasi-instantaneous jump when it is inward. The "time-independent" and the time-dependent currents may flow through different channels or through the same channels.

The time-dependent current on hyperpolarization is observed in the presence or in the absence of sodium, calcium, or chloride ions, is not suppressed by cobalt, diltiazem, or cadmium, but is suppressed by cesium and barium. The reversal potential of this current behaves like a potassium electrode, it must then be carried by potassium ions.

The kinetics of its activation are faster for more negative potentials, in the milliseconds range. The time constant logarithm is roughly linear with the potential. The potential necessary to increase tenfold the time constant is 80 to 100 mV, either in normal or isotonic K. This value is higher that what has been reported on skeletal muscle (Leech & Stanfield, 1981) and the egg of the starfish (Hagiwara & Yoshii, 1980), which reflects a weaker voltage-dependence. In their study, Hagiwara and Yoshii show that the voltage dependence of the time constant is lower when the temperature is increased and their data are consistent with the present results if we extrapolate to the mammalian physiological temperature.

The potassium channel blockers, cesium and barium, act differently on this current. Cesium at low concentrations slows down the activation of the channel, and finally blocks it. The blockage increases with hyperpolarization, and the percentage of block is a sigmoid function of potential, as already described on skeletal muscle (Gay & Stanfield, 1977), eggs (Hagiwara et al., 1976), and frog heart (Argibay et al., 1983). Conversely, barium has little or no effect on the activation kinetics of the channel, but promotes a time-dependent block resembling an inactivation. Similar effects have been described on skeletal muscle (Standen & Stanfield, 1978; DeCoursey & Hutter, 1984), eggs (Hagiwara et al., 1978) and on heart (DiFrancesco, Ferroni & Visentin, 1984).

As on egg cells (Hagiwara et al., 1976), skeletal muscle (Leech & Stanfield, 1981) and on single-channel recording on heart (Kurachi, 1985), the exponential time-dependent activation is preceded by a faster step or an "instantaneous" jump. When the current is outward, it also appears instantaneous. Although we could not accurately measure it, we got indirect information on its nature. This "instantaneous" current is resistant to cobalt, diltiazem, cadmium, and is blocked by cesium and barium. Its equilibrium potential is shifted like the potassium equilibrium potential, so it must be carried by potassium. The overall conductance curve of the membrane, relative to the sum of the time-dependent and the time-independent currents, is similar to the activation curve of the time-dependent current only. This suggests that the time-dependent activation of the current reflects the reopening of the channels which have been closed by a depolarizing prepulse, i.e. that the overall conductance and the time-dependent activation are related. The shape of the activation curve also indicates that the conductance is not fully deactivated at potentials positive to the potassium equilibrium potential, so that the outward time-independent current is likely flowing at least partly through the same channels as the inward current.

These experimental data suggest that the time-independent and the time-dependent currents are part of the same inward-rectifying potassium current, called I_{K1} on heart.

Our results indicate that the open-channel conductance must be constant for potentials negative to the potassium equilibrium potential, in agreement with studies on single channel (Kameyama, Kiyosue & Soejima, 1983; Sakmann & Trube, 1984*a*; Kurachi, 1985; Payet, Rousseau & Sauve, 1985).

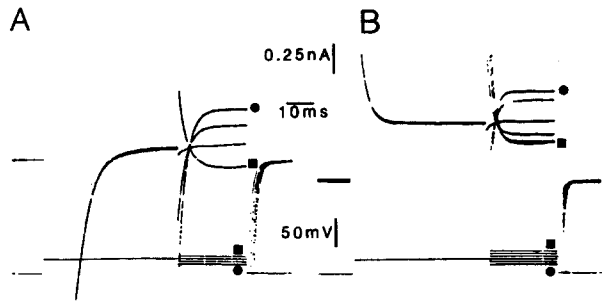

Fig. 7. Comparison of the currents recorded in the same cell in modified tyrode containing 5 mM Ca and in Na-free, Ca-free medium. The holding potential is −80 mV. (*A*) Currents in normal sodium, 5 mM Ca. The peak inward current during the prepulse is offscreen. The current at the end of the test pulse is net inward for the most positive potential (square). (*B*) Same protocol on the same cell after a change to Na- and Ca-free medium (with 5 mM Cd). The holding current is slightly inward. No fast sodium current is activated during the prepulse. Note the strong negative slope conductance in spite of the absence of inward current (same picture as Fig. 2)

This current plays a major role in the cardiac action potential, mainly on mammalian ventricle where the delayed outward potassium current is small (Boyett, Coray & McGuigan, 1980). Its role in the depolarization may be discussed. Because of the negative slope conductance, it is capable of inducing action potential-like responses in the absence of inward currents (Tourneur, 1986). We may question the importance of this negative slope conductance, relative to an inward current. The following experiment shows the currents recorded on the same cell in normal sodium and 5 mM calcium (Fig. 7*A*), and in the absence of sodium and calcium (Fig. 7*B*). When present, the fast sodium current is inactivated by a prepulse. In these conditions, in calcium-containing solution, the current measured during the second step has a negative slope conductance and gets net inward. In calcium-free, the current has a negative slope conductance, but is always outward. From −50 to −20 mV, the current increases in the inward direction by 550 pA in calcium, and by 400 pA in Ca-free. This result suggests that even in elevated calcium solution, the outward current is roughly responsible for $\frac{3}{4}$ of the negative slope conductance measured in calcium in this range of potential.

Conclusion

The inwardly rectifying potassium channel is present on many different preparations with similar characteristics. Its activation properties have been extensively studied on skeletal muscle and on eggs (for review, *see* Stanfield et al., 1980). The present study shows that the I_{K1} current activates with time when it is inward. The outward part of the current has a negative slope and decreases to zero with depolarization. The effects of cesium and of barium are similar on heart, skeletal muscle and eggs. The channels are therefore probably very close, if not identical, on these tissues.

On heart, this current is known to play an important role during the repolarization of the action potential. The present results show that its negative slope conductance is not negligible compared to the negative slope conductance of the calcium inward current. Its role during the onset of the action potential has to be studied.

Preliminary results have already been published (Tourneur, Mitra & Morad, 1984; Mitra, Morad & Tourneur, 1985). We wish to thank J. Rendt for his collaboration in the experiments, R. Bonvallet and C. Ojeda for careful reading of the manuscript and for their helpful comments, and J. Barbery for artwork. This study has been supported by grant INSERM C.R.E. 855021.

References

Adrian, R.H., Freygang, W.H. 1962. Potassium conductance of frog muscle membrane under controlled voltage. *J. Physiol. (London)* **163:**104–114

Argibay, J.A., Dutey, P., Ildefonse, M., Ojeda, C., Rougier, O., Tourneur, Y. 1983. Block by Cs of K current I_{K1} and of carbachol induced current I_{Cch} in frog atrium. *Pfluegers Arch.* **397:**295–299

Boyett, M.R., Coray, A., McGuigan, J.A.S. 1980. Cow ventricular muscle: 1. The effect of extracellular potassium concentration on the current-voltage relationship. 2. Evidence for a time-dependent outward current. *Pfluegers Arch.* **389:**37–44

Bustamante, J.O., Watanabe, T., McDonald, T.F. 1982. Nonspecific proteases: A new approach to the isolation of adult cardiocytes. *Can. J. Physiol. Pharmacol.* **60:**997–1000

Chesnoy-Marchais, D. 1983. Characterization of a chloride conductance activated by hyperpolarization in *Aplysia* neurones. *J. Physiol. (London)* **342:**277–308

DeCoursey, T.E., Hutter, O.F. 1984. Potassium current noise induced by barium ions in frog skeletal muscle. *J. Physiol. (London)* **349:**329–351

DiFrancesco, D., Ferroni, A., Visentin, S. 1984. Barium-induced blockade of the inward rectifier in calf Purkinje fibres. *Pfluegers Arch.* **402:**446–452

DiFrancesco, D., Ferroni, A., Visentin, S., Zaza, A. 1985. Cadmium induced blockade of the cardiac fast Na channels in calf Purkinje fibres. *Proc. R. Soc. (London) B* **223:**475–484

Gay, L.A., Stanfield, P.R. 1977. Cs^+ causes a voltage-dependent block of inward K currents in resting skeletal muscle fibers. *Nature (London)* **267:**169–170

Hagiwara, S., Miyazaki, S., Moody, W., Patlak, J. 1978. Blocking effects of barium and hydrogen ions on the potassium current during anomalous rectification in the starfish egg. *J. Physiol. (London)* **279:**167–185

Hagiwara, S., Miyazaki, S., Rosenthal, N.P. 1976. Potassium current and the effect of cesium on this current during anoma-

lous rectification of the egg cell membrane of a starfish. *J. Gen. Physiol.* **67**:621–638

Hagiwara, S., Takahashi, K. 1974. The anomalous rectification and cation selectivity of the membrane of a starfish egg cell. *J. Membrane Biol.* **18**:61–80

Hagiwara, S., Yoshii, M. 1980. Effect of temperature on the anomalous rectification of the membrane of the egg of the starfish, *Mediaster aequalis*. *J. Physiol. (London)* **307**:517–527

Hall, A.E., Hutter, O.F., Noble, D. 1963. Current voltage relations of Purkinje fibres in sodium-deficient solutions. *J. Physiol. (London)* **166**:225–240

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

Kameyama, M., Kiyosue, T., Soejima, M. 1983. Single channel analysis of the inward rectifier K current in the rabbit ventricular cells. *Jpn. J. Physiol.* **33**:1039–1056

Kandel, E.R., Tauc, L. 1966. Anomalous rectification in the metacerebral giant cells and its consequences for synaptic transmission. *J. Physiol. (London)* **183**:287–304

Katz, B. 1949. Les constantes électriques de la membrane du muscle. *Arch. Sci. Physiol. (Paris)* **3**:285–299

Kurachi, Y. 1985. Voltage dependent activation of the inward rectifier potassium channel in the ventricular cell membrane of the guinea-pig heart. *J. Physiol. (London)* **336**:365–385

Leech, C.A., Stanfield, P.R. 1981. Inward rectification, voltage and $[K]_o$. *J. Physiol. (London)* **319**:295–309

Maughan, D.W. 1976. Potassium movement during hyperpolarization of cardiac muscle. *J. Membrane Biol.* **28**:241–262

Mitra, R., Morad, M. 1985. A uniform enzymatic method for the dissociation of myocytes from heart and stomach of vertebrates. *Am. J. Physiol.* **249**:H1056–H1060

Mitra, R., Morad, M., Tourneur, Y. 1985. Time-dependent activation of the inward rectifier I_{K1} on guinea-pig cardiac cells. *J. Physiol. (London)* **358**:52P

Nakamura, Y., Nakajima, S., Grundfest, H. 1965. Analysis of spike electrogenesis and depolarizing K-inactivation in electroplaques of *Electrophorus electricus*. *J. Gen. Physiol.* **49**:321–349

Noble, D. 1984. The surprising heart: A review of recent progress in cardiac electrophysiology. *J. Physiol. (London)* **353**:1–50

Noma, A. 1983. ATP regulated K channels on heart muscle. *Nature (London)* **305**:147–148

Ohmori, H. 1978. Inactivation kinetics and steady-state current noise in the anomalous rectifier of tunicate egg cell membrane. *J. Physiol. (London)* **281**:77–99

Payet, M.D., Rousseau, E., Sauve, R. 1985. Single-channel analysis of a potassium inward rectifier in myocytes of newborn rat heart. *J. Membrane Biol.* **86**:79–88

Rougier, O., Vassort, G., Stämpfli, R. 1968. Voltage-clamp experiments on frog atrial heart muscle fibres with the sucrose gap technique. *Pfluegers Arch.* **301**:91–108

Sakmann, B., Trube, G. 1984*a*. Conductance properties of single inwardly rectifying potassium channels in ventricular cells from guinea-pig heart. *J. Physiol. (London)* **347**:641–657

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

Standen, N.B., Stanfield, P.R. 1978. Potential and time dependent blockade of inward rectification in frog skeletal muscle fibre by barium and strontium ions. *J. Physiol. (London)* **280**:169–191

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

Stanfield, P.R., Standen, N.B., Leech, C.A., Ashcroft, F.M. 1980. Inward rectification in skeletal muscle fibres. Molecular and cellular aspects of muscle function. *Adv. Physiol. Sci.* **5**:247–262

Tourneur, Y. 1986. Action potential-like responses due to the inward-rectifying potassium channel. *J. Membrane Biol.* **90**:115–122

Tourneur, Y., Mitra, R., Morad, M. 1984. Activation of the inwardly rectifying K^+ current in guinea-pig atrial and ventricular cells. *Biophys. J.* **45**:136a

Trautwein, W., McDonald, T.F. 1978. Current voltage relations in ventricular muscle preparations from different species. *Pfluegers Arch.* **374**:79–89

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