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Potassium currents in cardiac cells

by E. Carmeliet, G. Biermans, G. Callewaert and J. Vereecke

Laboratory of Physiology, University of Leuven, Gasthuisberg, Herestraat, B-3000 Leuven (Belgium)

Summary. The kinetic properties of the inwardly rectifying K current and the transient outward current in cardiac cells were investigated.
In sheep Purkinje fibers superfused with Na-free K-free solution, time-dependent changes in the conductance of the inward rectifier are described. In patch clamp experiments the inward rectifier inactivates during hyperpolarization, as can be seen by a decrease in the open state probability. Using whole cell clamp on ventricular myocytes it is demonstrated that the inactivation during hyperpolarization is due to blocking of the channel by external Na, Mg and Ca.
The channels responsible for the transient outward current in cow, sheep and rabbit Purkinje fibers are identified using single channel recording. It is demonstrated that in all three preparations the channels are K-selective. The channel in cow Purkinje cells has a large conductance and is regulated by voltage and internal Ca concentration. The channels identified in the sheep and rabbit cells have a much smaller conductance.
Key words. Heart; membrane; electrophysiology; K current; voltage clamp; single channel.

Introduction

A number of different K currents have been described in cardiac cells:
Cardiac cells at rest are mainly K-selective, so that the membrane potential is largely governed by the K equilibrium potential. The channel responsible for the K-selectivity of the membrane was first described as a purely voltage-dependent channel which passes inward current more easily than outward current[19]. It has been called the inwardly rectifying K current (i_{K1}). This current is prominent in ventricular cells. It is present to a lesser extent in atrial cells, and its amplitude is very small in the sino-atrial node.
A transient outward current (i_{to}) has been found in sheep[27] and cow Purkinje fibers[31], and in rat ventricular cells[12]. This current causes a fast repolarization to the plateau level in Purkinje cells, and contributes to the final repolarization in the rat ventricular cells.
Another current, the delayed outward current, affects the duration of the plateau. It was first described in Purkinje fibers and called i_{x1}[22], later it was renamed i_x. In ventricular cells a delayed outward current has been described, and identified as carried by K; therefore it was named i_K[20]. The same terminology is now also often applied for the delayed outward current in Purkinje cells, since deviation of its reversal potential from the K equilibrium potential appears to be related to K accumulation in the narrow clefts between the cells.

Acetylcholine (ACh) exerts its chronotropic effect by increasing the K current flowing through the membrane[33]. The ACh-sensitive K channel shares a number of properties with i_{K1}[9]. However its gating and conductance properties and its sensitivity to Ba are different from those of i_{K1}[5,24,28]; therefore it must be considered to be a different channel and it is called $i_{K(ACh)}$.

Metabolic inhibition is known to shorten the cardiac action potential, and its effect is mediated by an increase of outward K current[11,36]. In patch clamp experiments an ATP-sensitive large conductance K channel has been identified which is controlled by the intracellular ATP level[23,34]. In normal conditions this channel is closed. However, inhibiting the oxidative metabolism or lowering the ATP level in the cell causes the channel to produce frequent openings. Its large conductance makes it clearly distinct from the other K channels and the channel will be called $i_{K(ATP)}$.

A K channel which is sensitive to intracellular Na ions, $i_{K(Na)}$, has also been described[14]. Its function remains unclear, but it may play a role in controlling the cell homeostasis in situations where the intracellular Na is elevated.

A number of problems about the kinetics of these channels remain unsolved. The aim of this work was to investigate possible gating properties of the inward rectifier, to identify the channels responsible for the transient outward current, and to study the mechanisms which control their gating.

Results

The inwardly rectifying potassium current

The inwardly rectifying K current in cardiac tissue (i_{K1}) has been regarded as time-independent, since for moderate voltage steps this current remains constant during the voltage step. Decay of i_{K1} was nevertheless observed during hyperpolarizing voltage steps in sheep Purkinje fibers[1]. This decay was not attributed to kinetic properties of the channel, but to changes in K equilibrium potential due to K depletion in the narrow spaces between the cells. Time-dependent changes in the conductance of the inward rectifier have been described, however, in other preparations, such as egg cells[10,25] and frog sartorius muscle[32]. We therefore wanted to investigate whether time-dependent conductance changes are also present in the inwardly rectifying K current in cardiac cells.

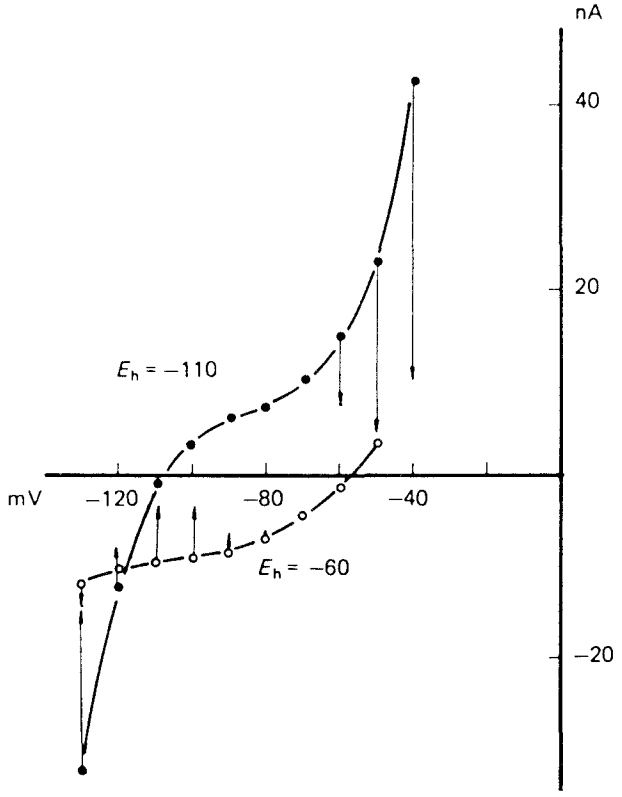

Figure 1. Instantaneous current-voltage relations obtained from a holding potential of −110 mV (●) and −60 mV (○) in a sheep Purkinje fiber superfused with K-free, Na-free solution. The arrows indicate the time-dependent changes during a 10 s clamp.

The inwardly rectifying K channel in cardiac cells has a genuine gating mechanism

Time-dependent conductance changes of the inward rectifier in sheep Purkinje fibers. Slow time-dependent changes which could not be explained solely on the basis of limited extracellular K diffusion have been obtained in sheep Purkinje fibers superfused with a Na- and K- free Tyrode solution[4]. (Na was replaced by Tris or choline).

The starting point of the investigation was the finding that the preparation could have two different stable resting potentials. The resting potential has a value near −100 mV if Na is removed before K, while a low resting potential near −50 mV is obtained when K is removed first. A shift from the highly negative resting potential to the low resting potential can be obtained by applying depolarizing clamp steps of a certain minimum amplitude and duration. The inverse change can be obtained with hyperpolarizing clamps. The change in resting potential occurs in an all-or-nothing fashion, dependent of the voltage or the duration of the step[4].

Characterization of the current responsible for this process was obtained by applying voltage steps to different potentials, either from a holding potential of −110 mV or from −60 mV, in a preparation superfused with Na-free K-free solution. Slow time-dependent changes of the ionic current are seen during the clamp steps and the instantaneous current is strongly dependent on the holding potential in these conditions. The results are summarized in figure 1.

When the holding potential was at −60 mV, the instantaneous current-voltage relation is fairly linear at potentials negative to −80 mV. Clamp steps to a potential at −70 or −80 mV resulted in an inward current which is time-independent. At potentials between −90 and −120 mV, the inward current decreases with time (as indicated by the arrows), while an increase with time is seen for steps negative to −120 mV, suggesting a genuine reversal potential.

When the holding potential was at −110 mV, the instantaneous current-voltage relation showed pronounced inward rectification. In this condition a decrease in outward current can be observed for potentials positive to −55 mV.

Several findings indicate that the effect is due to i_{K1}:

1) Conductance measurements during a hyperpolarizing clamp step from −40 to −95 mV indicate that the time-dependence is due to an increasing outward current, and therefore demonstrate that the time-dependent changes are due to a channel with a very negative equilibrium potential, which leaves K channels as the only candidates.

2) The difference between the two instantaneous current-voltage relations shows clear inward rectification and has a reversal potential at −115 mV. This reversal potential is close to the potential at which the current during clamps from −60 mV reverses direction, and is likely to correspond to the K equilibrium potential assuming a K concentration of about 1 mM in the intercellular clefts.

3) The time-dependent current and the difference current between the two instantaneous current-voltage relations are greatly reduced by application of 0.3 mM Ba (which is known to block i_{K1}).

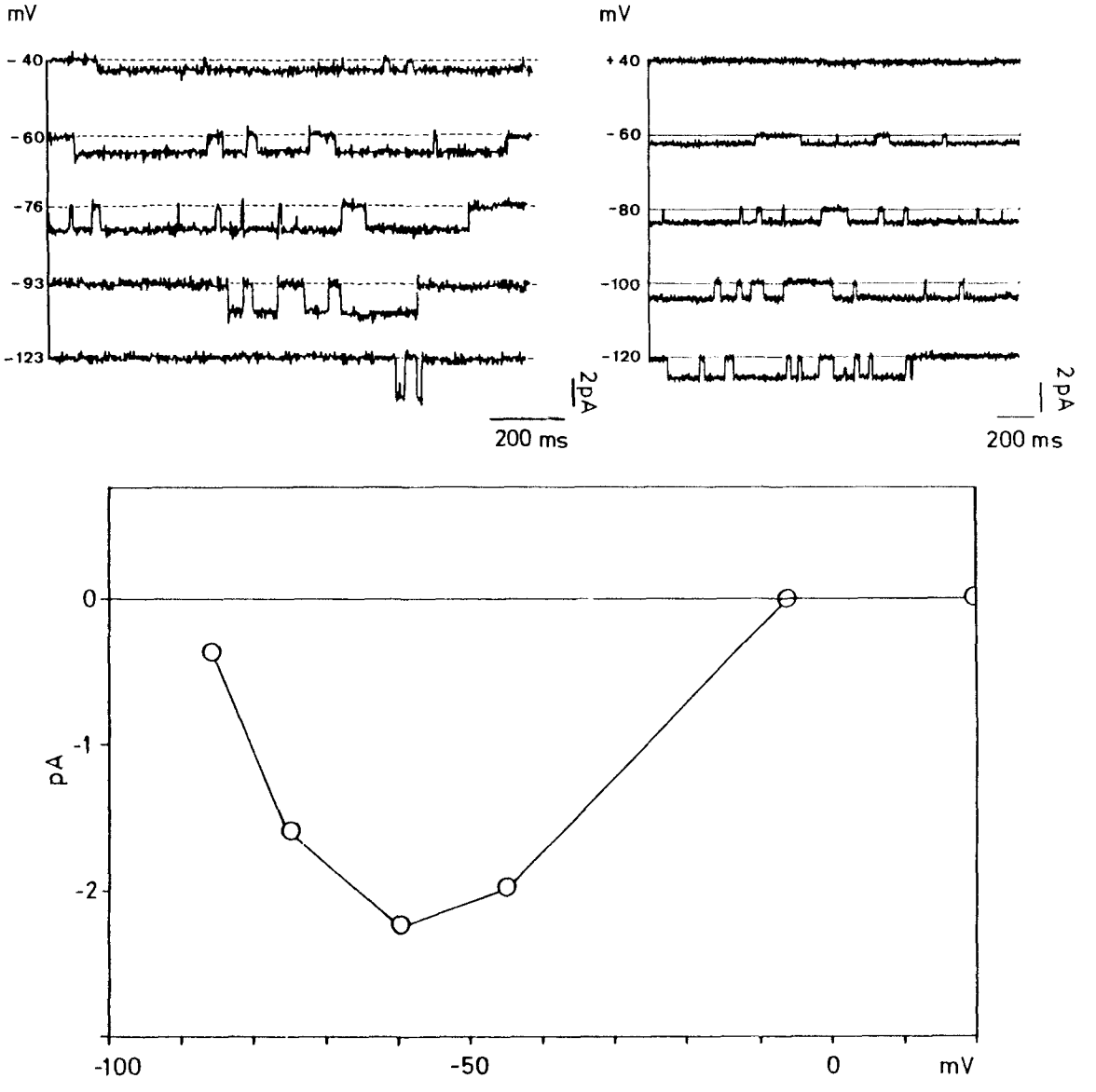

Figure 2. Top: Single channel currents flowing through the inwardly rectifying K channel at different potentials in a cell-attached patch of a sheep Purkinje cell. In the upper left panel the pipette contained 140 mM KCl, while the currents in the right panel were obtained in 70 mM KCl. Bottom: voltage-dependence of the mean current with 140 mM KCl in the pipette.

These findings strongly imply that the effects observed in Na-free K-free medium are mediated by i_{K1}. Since in these conditions the instantaneous current-voltage relation measured from a holding potential at −50 mV is similar to the one obtained when the inward rectifier is blocked by Ba, it appears that the inward rectifier channels are closed when the membrane is at the low resting potential. Holding the membrane for a minimum period of time at more negative potentials appears to activate the channels.

The phenomenon cannot be explained solely by accumulation and depletion of K in the extracellular spaces between the cells.

a) Depletion is characterized by a decreasing inward current. In contrast, the time-dependent current becomes more inward as a function of time during clamp steps from the low negative resting potential to strongly negative potentials (see e.g. fig. 5 in Carmeliet[4]).

b) Depletion during a hyperpolarizing clamp can never result in a change from net inward to net outward current (since this would cause K accumulation instead). However, during clamps from −50 mV to a potential slightly positive to the high negative resting potential, the current, which is initiallly net inward, becomes net outward within a few seconds (see e.g. fig. 10 in Carmeliet[4]).

The phenomenon cannot be explained solely by purely voltage-dependent Markow processes.

a) The existence of two stable levels of resting potential depending of the previous history and of steady-state current-voltage relations dependent on the holding potential, exclude memoryless processes.

b) In a cell which had a stable potential at −97 mV and another at −60 mV, the membrane was clamped for several minutes to a holding potential of −80 mV before applying pulses to different test potentials. In one case the membrane was allowed to stay at the resting potential at −60 mV before clamping to the holding potential, while in the other case the holding potential was reached from the highly negative resting potential at −97 mV. Although in both cases the mem-

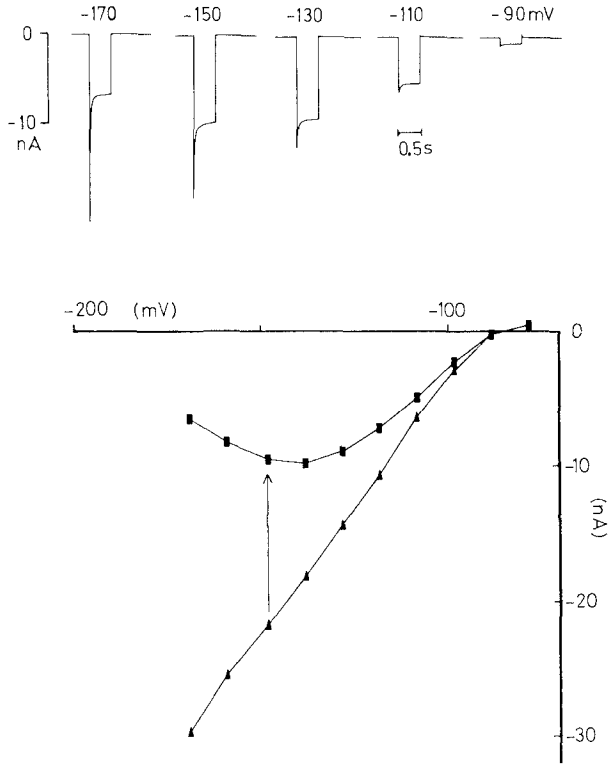

Figure 3. Inactivation of the inward rectifier in guinea pig ventricular myocytes in normal Tyrode solution. Upper half: ionic currents during large hyperpolarizing voltage steps. Lower half: current-voltage relation of the peak inward current (▲), and of the steady-state current (■).

brane was clamped to the same holding potential for several minutes, which allowed the currents to reach a steady-state, inward rectification was only present when the holding potential had been reached from the highly negative resting potential. It was absent when the membrane had been at the low resting potential prior to clamping to the holding potential (see e.g. fig. 12 in Carmeliet[4]). Therefore the difference between the two instantaneous current-voltage relations in figure 1 is not caused by a different holding potential, but must be explained by assuming hysteresis.

The phenomenon is sensitive to small changes in the extracellular K concentration. The addition of 0.54 mM K to the solution causes a stabilization of the membrane potential at the more negative level. In this case no second stable potential level can be obtained, and the instantaneous current-voltage relation always shows substantial inward rectification.

The results can be explained by assuming an interaction between K and a binding site in the membrane. Occupancy of the site by K is assumed to open the i_{K1} channel. When the resting potential is low, the occupancy of the site by K is low. Hyperpolarization increases the occupancy, and therefore promotes the activation of the channel. When the potential during the hyperpolarizing step is positive to E_K, outward current will flow through the channel, causing a rise of the K concentration in the intercellular clefts. This increased K concentration will also enhance the occupancy of the site, and will thus act as a positive feedback mechanism, stabilizing the channel in the 'open position'. Depolarization will decrease the occupancy of the site by K, and will close the channel. The resulting lack of outward K current will cause a lower K concentration in the clefts, which stabilizes the channel in the closed position. Therefore a combination of voltage-dependent binding of K to a site controlling the channel gating, and changes in external K concentration due to accumulation-depletion, can explain the two stable levels of membrane potential in Purkinje fibers superfused with K-free, Na-free solution.

Inactivation of the inward rectifier in single channel recordings in Purkinje cells. The interpretation of the findings in Purkinje fibers is complicated by the multicellular nature of the preparation, since accumulation and depletion of K affect the process. Single channel studies are free of these artefacts and could therefore provide more direct evidence for channel gating.

Channel openings corresponding to the inward rectifier in cell-attached patches of sheep Purkinje cells are shown in the upper half of figure 2. The left side illustrates the currents obtained with 140 mM KCl in the pipette, while at the right side the currents recorded in 70 mM KCl are illustrated. In 140 mM K the single channel current is −1.3 pA at −60 mV, and it becomes smaller for less negative potentials. When the concentration of K in the pipette is changed by partially replacing K by Na, the currents become smaller (upper right). The single channel current-voltage relation in 140 mM K has an extrapolated reversal potential near 0 mV, and when the K concentration in the pipette is changed, a shift of the reversal potential is observed as expected for a K electrode, which enables us to identify the channels as K channels. At negative potentials the channel has a single channel conductance of 27 pS in 140 mM K_o, and 18 pS in 70 mM K_o. When the patch is depolarized to potentials positive to the K equilibrium potential, no outward currents can be found (see fig. 2 upper right, trace labeled +40 mV). The channel therefore shows strong inward rectification and therefore appears to correspond to i_{K1}. The properties of the channel are the same as these of the channel found in ventricular myocytes by Sakmann and Trube[29, 30], who identified the channel as i_{K1}.

These channels are controlled by a potential-dependent gating mechanism. Hyperpolarization of the patch membrane causes a reduction of the number of opening transitions per unit time and of the average channel open time, together with an increase of the mean closed time. These changes cause a decrease of the open state probability for more negative potentials. The channels are thus controlled by voltage-dependent gates. Multiplying the single channel current with the open probability of the channel results in the mean current. When the mean current is plotted as a function of the membrane potential, a curve is obtained which shows a region of negative slope for strong hyperpolarizations (fig. 2, bottom). Similar findings have been obtained in ventricular myocytes by different authors[13, 17, 26, 29].

Inactivation of the inward rectifier during hyperpolarizing pulses in single cells. Single channel experiments provided direct evidence for a voltage-dependent gating mechanism. However, single channel recordings of the inwardly rectifying K channel under physiological conditions are difficult to obtain, owing to the small size of the single channel currents. Therefore we used the whole cell clamp technique to study the kinetics of the inward rectifier under more physiological conditions. As a preparation we chose single ventricular myocytes isolated from guinea pig hearts. In order to be able to inject large currents without causing artefacts produced by series resistance in the pipette, we used a two electrode whole cell clamp technique. Both electrodes were suction pipettes and were filled with a solution containing 140 mM KCl, 6 mM $MgCl_2$, 154 μM $CaCl_2$, 5 mM Na, ATP and 5 mM EGTA resulting in a pCa of 8. The solution was buffered with 10 mM Hepes and brought at pH 7.2 using KOH. The experiments were performed at room temperature.

The upper part of figure 3 shows the membrane currents in normal Tyrode solution during 500 ms hyperpolarizing voltage steps from a holding potential at −80 mV. All currents

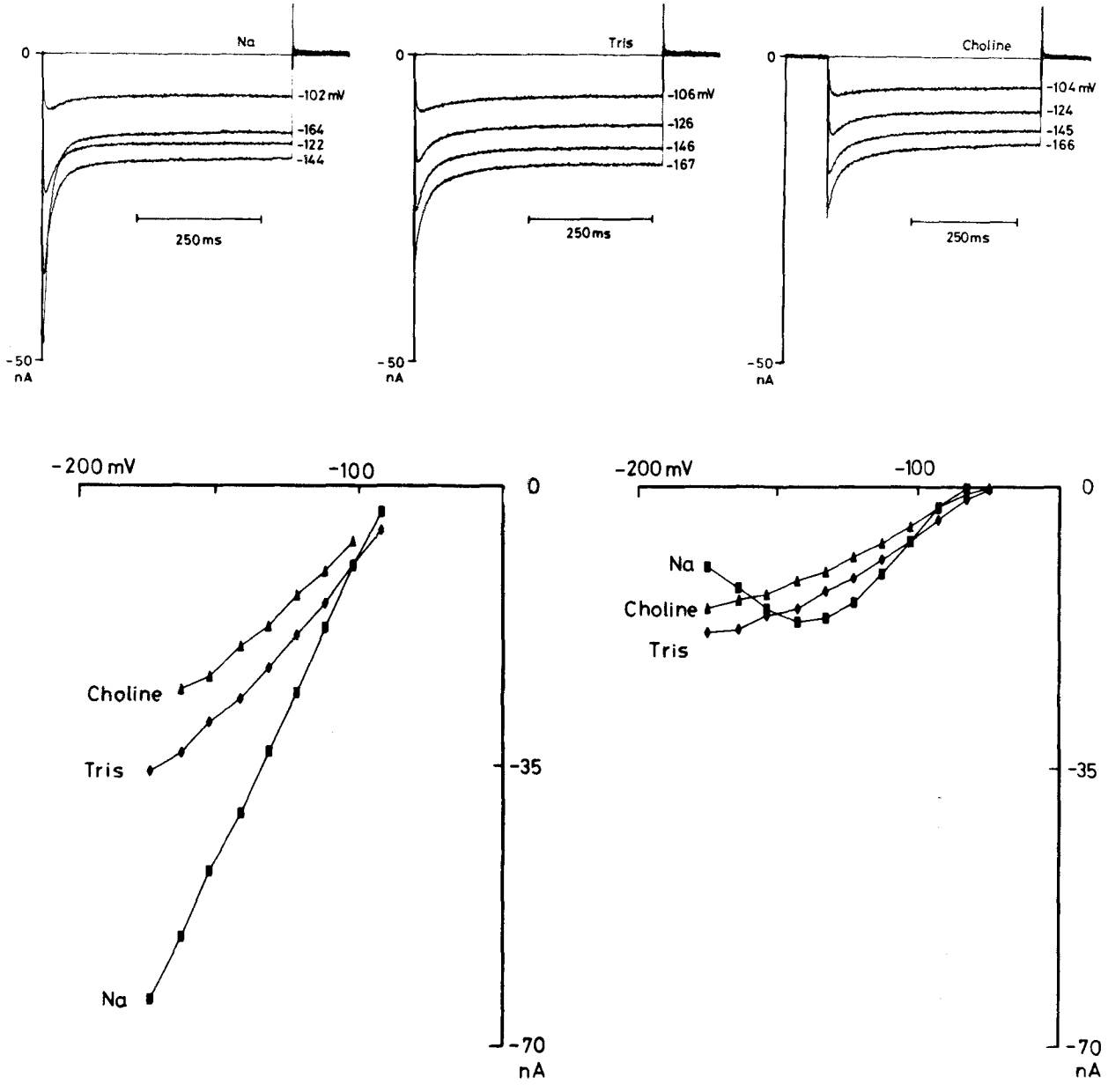

Figure 4. Effect of Na substitution on the inward rectifier in a guinea pig ventricular myocyte. Upper half: the currents recorded during strong hyperpolarizing pulses from a holding potential at −75 mV, in normal Tyrode solution (left), and when Na was replaced by either Tris (middle) or choline (right). Lower half: current-voltage relation of the peak inward current (left) and the steady-state current (right) with Na, and when Na was replaced by Tris or choline.

show a time-dependent decline. The amplitude of the decline is small for a step to −90 mV but increases markedly for more negative steps. The peak current becomes more inward for larger hyperpolarizations, in contrast to the steady-state current which decreases for very large test pulses. The peak and the steady-state current-voltage relation are shown in the lower part of the figure. The peak current-voltage relation is almost linear while the steady-state current-voltage relation has a region of negative slope at potentials negative to −140 mV. Such a region of negative slope cannot be explained by depletion-accumulation, and must therefore be due to a gating mechanism.

Inactivation of the inward rectifier during hyperpolarization is due to blocking by external ions

Inactivation by external Na ions. The decline of the current during strong hyperpolarizations and the negative slope in the steady-state current-voltage relation could be due to a purely voltage-dependent gating mechanism, or to a voltage-dependent block by the extracellular Na ions, as has been found in egg cells[25]. Therefore we compared the currents measured in single ventricular myocytes in normal Tyrode solution and in solutions where the Na was replaced by Tris and choline.

When Na is replaced by Tris, a large reduction of the peak inward current is seen (fig. 4). Also the time-dependent decay of the current is diminished. Furthermore, while in the presence of Na the steady-state current at −164 mV is smaller than at −144 mV, this is not the case in the presence of Tris. Substituting choline instead of Tris for Na results in similar effects, but the currents are somewhat smaller.

Figure 4 shows the peak and the steady-state current-voltage relations in the presence and in the absence of Na. It is clear that upon replacement of the extracellular Na ions the negative slope region in the steady-state current-voltage relation is removed. Na therefore causes a block of the inward rectifier and the block is much more pronounced at −164 than at − 102 mV. These findings indicate that Na ions block the

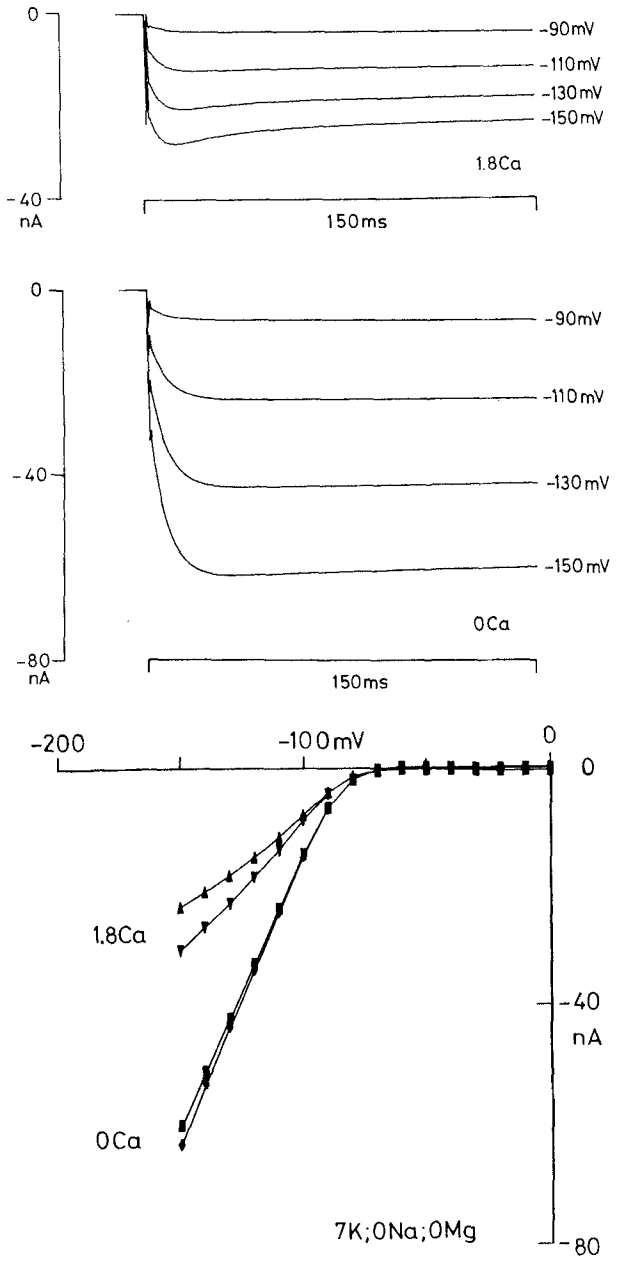

Figure 5. Blocking effect of external Ca ions on the inward rectifier. The preparation is a guineapig ventricular myocyte superfused with Mg-free solution in which Na was replaced by 245 mM sucrose. The solution contains 7 mM K. Top: currents in response to different hyperpolarizing clamp steps from a holding potential at −75 mV in the presence of 1.8 mM Ca. Middle: currents in Ca-free 0.5 mM EGTA containing solution. Bottom: peak (▼) and steady-state (▲) current-voltage relation in Ca-containing, and peak (♦) and steady-state (■) current-voltage relation in Ca-free solution.

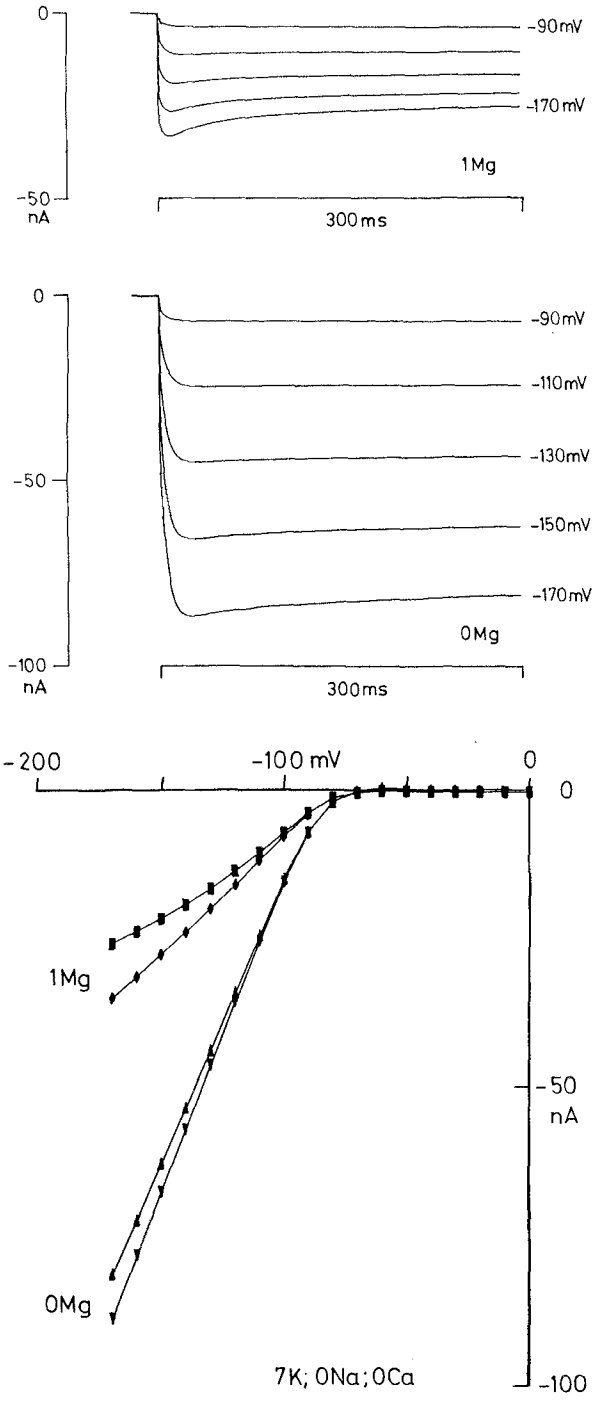

Figure 6. Blocking effect of external Mg ions on the inward rectifier. The preparation is a ventricular myocyte superfused with Ca-free, 0.5 mM EGTA containing solution, in which Na was replaced by 245 mM sucrose. The solution contains 7 mM K. Top: currents in response to different hyperpolarizing steps from a holding potential at −75 mV in the presence of 1 mM Mg. Middle: currents in Mg-free solution. Bottom: peak (♦) and steady-state (■) current-voltage relation in Mg-containing, and peak (▼) and steady-state (▲) current-voltage relation in Mg-free solution.

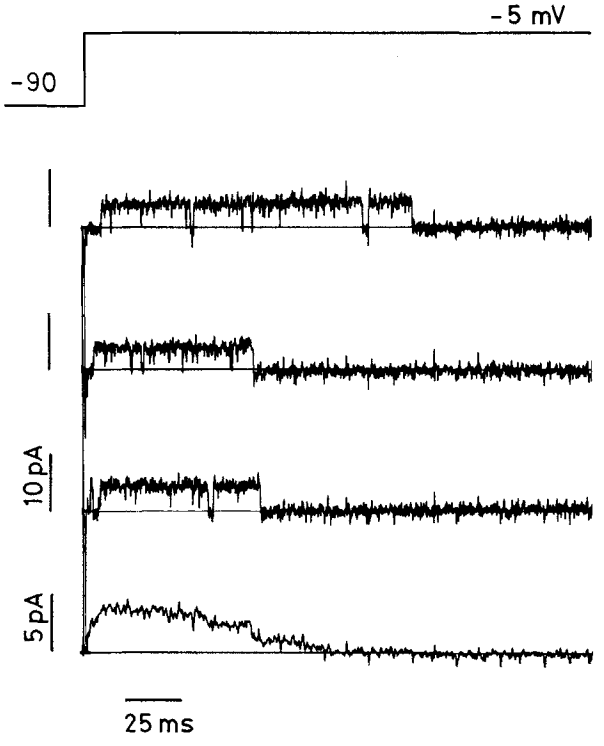

Figure 7. Channel openings during a depolarizing step in an outside-out patch from cow Purkinje cells. The pipette contained 140 mM KCl and 10^{-6} M $CaCl_2$. The bathing solution contained 32.5 mM KCl and 1.8 mM $CaCl_2$. Leakage and capacitative components were subtracted. The bottom trace contains the mean current of 100 clamp steps.

inward rectifier in a voltage-dependent way in ventricular cells, as has also been described in other preparations.

Besides the removal of the negative slope, a reduction of the currents in the positive slope region is also observed upon replacement of the Na ions. A similar reduction of the conductance is also found in the peak current-voltage relations. These phenomena can be interpreted as a facilitating effect of Na ions[25], or as an inhibitory effect of the substituting ions. Because the decline of the conductance is different in choline from that in Tris, we attribute the decline of conductance upon removal of extracellular Na to a blocking effect of the substitutes. Our data indicate that the block by choline or Tris is less voltage-dependent than the block by Na.

Inactivation by bivalent ions in the external solution. Since it is known that not only monovalent ions but also divalent ions block the inward rectifier, we investigated the effects of extracellular Ca and Mg.

The effect of Ca is illustrated in figure 5. The external solution contained 7 mM K, was Mg-free, and Na was replaced by sucrose in order to exclude any blocking effect of monovalent cations. The top part of the figure illustrates the currents obtained in the presence of 1.8 mM Ca. In the middle part the currents in the absence of Ca are shown (0.5 mM EGTA was used to chelate Ca). In the presence of Ca, hyperpolarization results in time-dependent inward currents, which show activation followed by a decay. Removing Ca from the external solution causes a pronounced increase of the inward currents, while the decay is absent. The bottom part of the figure shows the current-voltage relation of the peak inward current and the steady-state current in the presence and absence of Ca. These experiments demonstrate that Ca blocks the inward rectifier. The voltage-dependence of the block caused by divalent ions is less pronounced than that brought about by Na. Recently, in other experimental conditions, a Ca block of the inward rectifier has also been found by Mitra and Morad[21].

Figure 6 shows the effects of Mg removal on the inward rectifier in a cell which was superfused with a Na-free Ca-free solution containing 245 mM sucrose. The effects of Mg are qualitatively similar to the effects of Ca. However, Mg is about twice as potent as Ca for blocking the inward rectifier. These experiments show that the inward rectifier is controlled by a gating mechanism which depends on cations in the external solution. The inactivation which occurs during strong hyperpolarizations is largely due to external Na ions. Ca and Mg in the external solution also produce a marked block. This block by divalent ions is already important at potentials near the cell's normal resting potential, and is less voltage-dependent for potentials negative to −80 mV than is the block by Na. When Na, Ca and Mg are removed from the external solution, the inward rectifier can carry very large inward currents, which do not show a measurable decay. Since K depletion is known to cause decaying inward currents during hyperpolarizing steps, these experiments provide strong evidence that under normal conditions the K currents in single ventricular myocytes are not appreciably distorted by K depletion and accumulation.

The transient outward current

The transient outward current (i_{to}) has been described in different cardiac cells, such as sheep Purkinje fibers[7], cow Purkinje fibers[31], and rat ventricular cells[12]. The ionic nature of the current flowing through the channel has been a matter of controversy. Originally it was thought that the channel was a Cl channel[8]. However, strong evidence in favor of K ions as charge carriers has been presented[15, 16, 18, 35]. It has been argued that this current in cow Purkinje fibers is carried by K ions, and that the channel is activated by intracellular Ca[31]. The transient outward current in sheep Purkinje fibers is composed of two different components[6]. A brief outward component appears to be Ca-dependent and can be blocked by application of caffeine. A long-lasting component can be blocked by 4-aminopyridine and is not influenced by Ca. The channels responsible for these transient outward currents have not been identified in single channel studies. Therefore the ionic nature of the current, and the regulation of the kinetics of the transient outward current channel(s) will be studied using single channel recording.

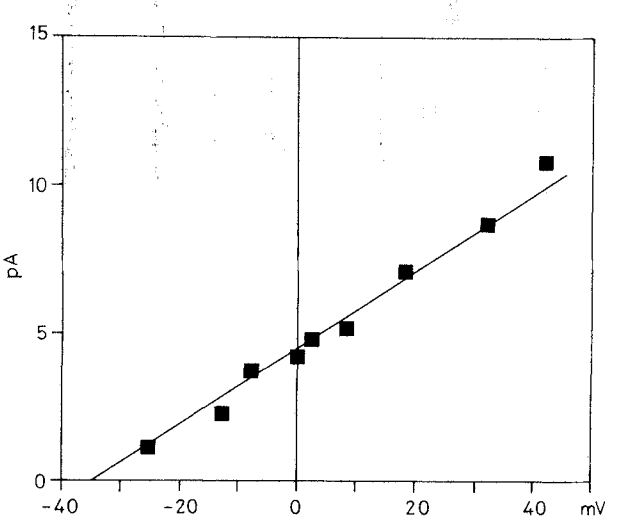

Figure 8. Current-voltage relation of the single channel current from a outside-out patch in cow Purkinje cell (same experiment as in fig. 7). With 140 mM K in the pipette and 32.5 mM K in the bathing solution the reversal potential was −35 mV and the single channel conductance was 130 pS.

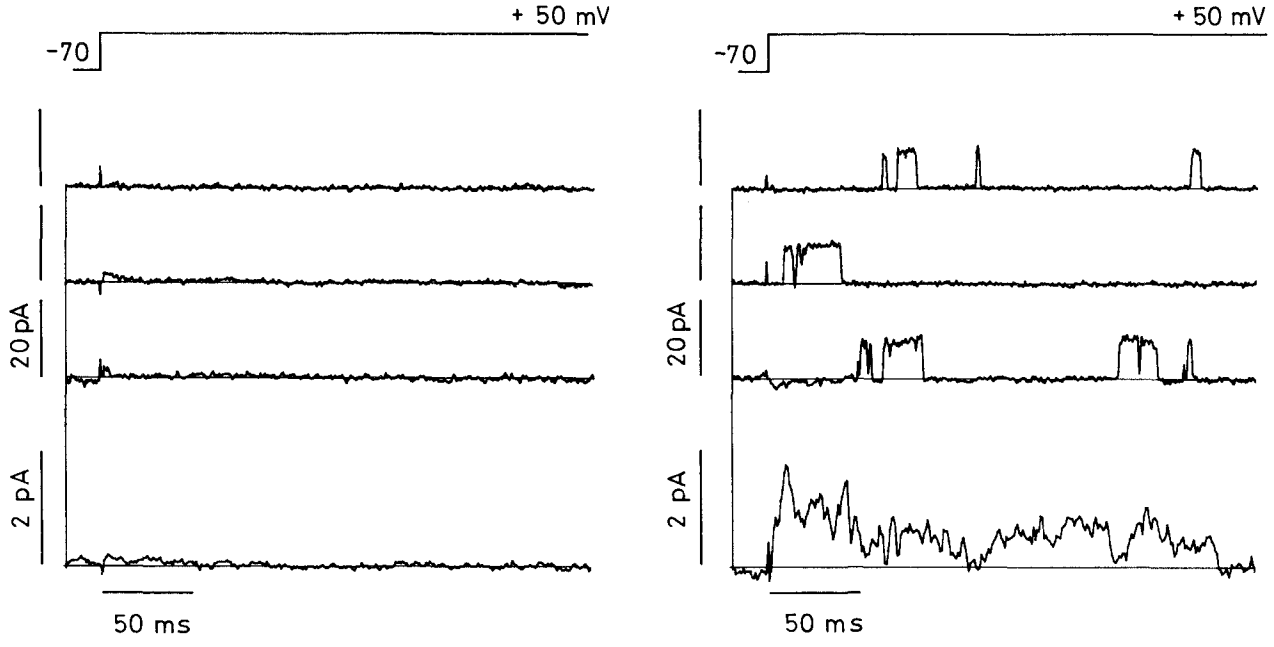

Figure 9. The effect of changes in Ca concentration at the cytoplasmic site of the membrane on the transient outward current channels in an inside-out patch from cow Purkinje cell. The upper trace shows the voltage step, the middle traces the single channel current and the lower trace the mean current from 50 clamp steps. The pipette contained 140 mM KCl and 1.8 mM $CaCl_2$. In the left half of the figure the bathing solution contained 10^{-9} M $CaCl_2$ and 140 mM KCl while in the right half 7×10^{-7} M $CaCl_2$ and 140 mM KCl was used.

The transient outward current channels in cow Purkinje cells. Single channel currents and mean current. Figure 7 shows single channel currents from an outside-out patch from a cow Purkinje cell, during depolarizing voltage steps from a holding potential at −110 mV. The patch pipette contained 140 mM KCl and 10^{-6} mM $CaCl_2$. The bathing solution contained 32.5 mM KCl and 1.8 mM $CaCl_2$. The experiments were done at room temperature. No channel openings can be seen at the holding potential. However, single channel activity is apparent immediately after the depolarizing step. The number of openings decreases as a function of time during the step, and no channel openings are found near the end of the step. The lower part of the figure shows the ensemble average of a large number of steps. This mean current reflects the time course of the average current flowing through a large number of channels as seen in classical voltage clamp experiments. The average current during the step is biphasic. A fast rising phase is followed by a slower decay

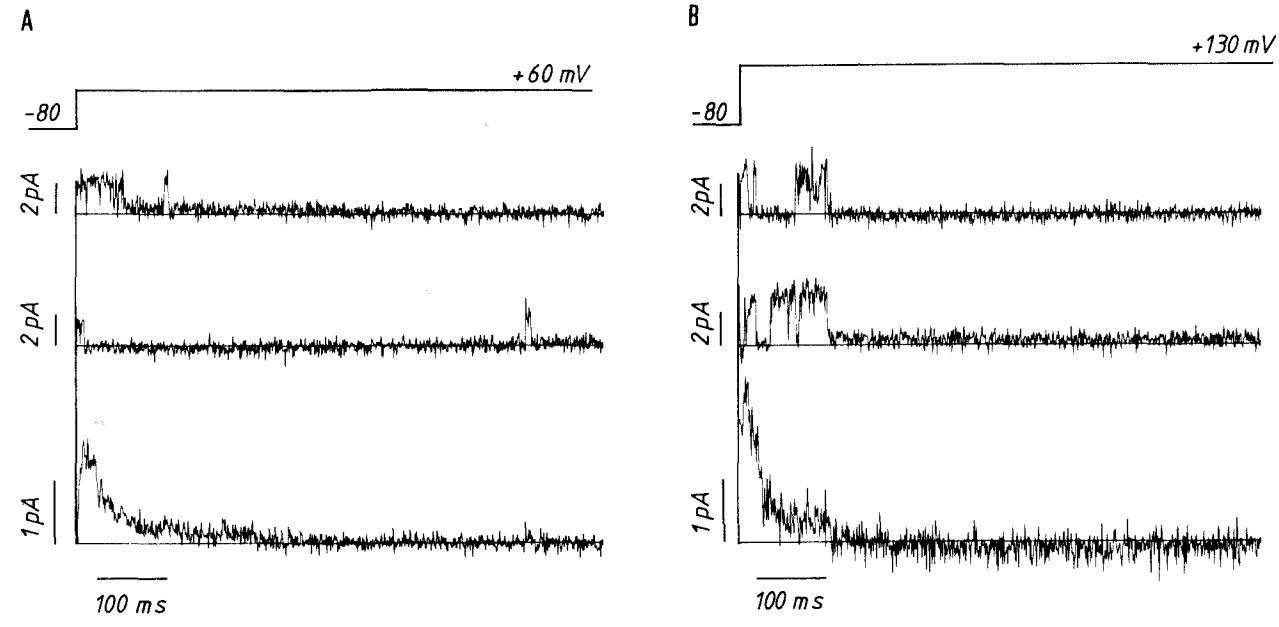

Figure 10. Channel openings during a depolarizing step to +60 mV (A) and to +130 mV (B) in a cell-attached patch from sheep Purkinje cells. The pipette contained 70 mM KCl and 1.8 mM $CaCl_2$. Leakage and capacitative components were subtracted. The bottom traces contain the mean current of 70 clamp steps.

with time constants between 20 and 100 ms. Since the steady-state current is zero, the decay is complete. The time course of the current thus corresponds to the time course of the transient outward current.

Channel conductance and selectivity. The single channel current in cow Purkinje cells is a linear function of the membrane potential at potentials between −25 and + 45 mV (fig. 8). Channel openings at potentials where the currents are inward are exceedingly rare. However, in the few cases were such events were seen, it appears that the linearity of the single channel current-voltage relation extends to strong negative potentials. The reversal potential of the current equals −35 mV in 32.5 mM K_o which is near the K equilibrium potential. The slope conductance equals 130 pS in this condition.

Experiments with inside-out and outside-out patches using different external K concentrations show a marked dependence of the reversal potential and of the single channel conductance on the extracellular K concentration. The shift of the reversal potential with external K indicates that the channel is selective for K ions. The single channel conductance increases with external K concentration. With 140 mM K in the pipette the conductance equals 200 pS. The conductance decreases to 140 pS in 70 mM K and to 120 pS at 10.8 mM K (see fig. 1 in Callewaert et al.[3]).

Ca-dependence of channel activity. The frequency of the opening events in cell-attached patches is generally rather small. There can be considerable variability from cell to cell, but the high frequency of channel openings as seen in figure 7 was exceptional. In general an important increase of the open probability was found when the pipette was moved away from the cell to yield an inside-out patch with the cytoplasmic side of the membrane exposed to 10^{-6} M Ca.

To determine whether changes in Ca activity at the cytoplasmic side of the membrane were responsible for the increase in the number of opening events, experiments were performed with inside-out patches with different Ca concentrations in the bathing solution. When the Ca activity at the cytoplasmic side is 10^{-9} M, channel openings are virtually absent during the voltage steps (fig. 9, left panel). Increasing the Ca to 7×10^{-7} M results in more frequent opening of the channel, while the single channel current remains unchanged. The mean current indicates a larger open-state probability of the channel (fig. 9, right panel). Also, the threshold for activation of the channel is lower (not shown).

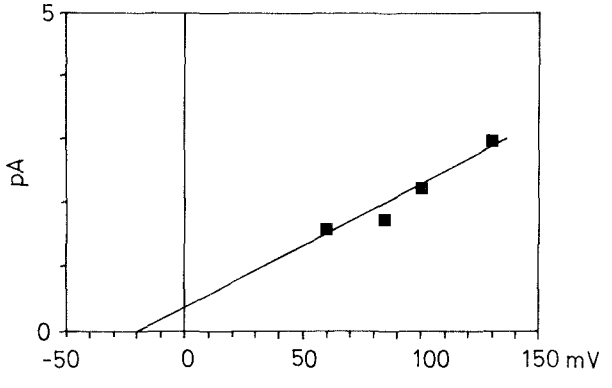

Figure 11. Current-voltage relation of the single channel current from a cell-attached patch isolated from a sheep Purkinje cell (same experiment as in fig. 10). With 140 mM K in the pipette and 70 mM K in the bathing solution, the reversal potential was −20 mV and the single channel conductance was 17 pS.

The transient outward current in sheep and rabbit Purkinje cells. In figure 10 transient outward current channels and the reconstructed mean currents in a cell-attached patch of a sheep Purkinje cell are shown. The pipette contains 70 mM K and the membrane is clamped from −80 mV to +60 mV (A) or to +130 mV (B). It is clear from this figure that the amplitude of the current flowing through the transient outward current channel in sheep is small as compared with the one shown in figure 7 for cow Purkinje cells.

The voltage-dependence of the single channel current in sheep Purkinje cells is shown in figure 11. The relation is linear with an extrapolated reversal potential at −20 mV, in agreement with the expected K equilibrium potential. The single channel conductance amounts to 17 pS with 70 mM K in the pipette.

In rabbit Purkinje cells the transient outward current channel has similar properties to the one in sheep Purkinje cells. The single channel conductance equals 26 pS at 36 °C with 70 mM K in the pipette.

From these data it can be concluded that the transient outward current channels which we found in the sheep and rabbit Purkinje cells appear to be similar; however, their single channel conductance differs greatly from the one in cow Purkinje cells.

Conclusions

Gating mechanisms different from purely voltage-dependent Hodgkin-Huxley mechanisms were described for two types of cardiac K channels. Using different preparations and techniques, it was demonstrated that the inwardly rectifying K channels are controlled by a gating mechanism. Using ventricular myocytes, we demonstrated that time- and voltage-dependent inactivation during hyperpolarization can be caused by binding of external Na, Ca or Mg to the channel. In Purkinje fibers it was shown that binding of a site with external K enables permeation of K ions through the i_{K1} channels. Since the inward rectifier does not appear to be different in Purkinje cells and in ventricular cells, it is tempting to speculate that this effect is due to an exclusion of blocking ions from the pore by K.

The transient outward current channel is controlled by an activation and an inactivation gate. It was demonstrated that in cow Purkinje cells the activation gate is controlled by voltage as well as internal Ca. Increasing Ca_i lowers the threshold and enhances the probability of the open state of the activation gate, and therefore appears to shift the voltage-dependence of the activation gate. Inactivation proceeds to completeness even at elevated Ca concentration, and therefore the inactivation seems to be primarily controlled by voltage. We also identified a channel carrying transient outward current in sheep and rabbit Purkinje cells which is different from the one in the cow.

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