Two Types of Single Inward Rectifying Potassium Channels in Rat Myocardial Cells

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Abstract. The patch-clamp method was used to examine inward rectifying potassium channels in the membrane of rat ventricular myocytes. Two types of inward rectifying channels strongly selective for K^+ ions and with different conductance and kinetics coexist in rat myocardial cells. When the concentration of K^+ was 140 mmol/l on the extracellular side of the patch, the conductance was 38.9 pS for type I channels and 25.7 pS for the type II. The type II channels had a detectable conductance (4 pS) at potentials positive than the potassium equilibrium potential. The mean open time was 18 ms at −60 mV patch membrane potential and 12 ms at −100 mV for type I channels, and 1.3 s at −60 mV and 0.94 s at −105 mV for type II channels, respectively. The opening probability of type II channels decreased with hyperpolarization. The type II channels can adopt several (about 10 or more) conductance states, which can occur either within one opening or as individual events.

Key words: Patch-clamp method — Rat myocardial cells — Single K^+ inward rectifying channels — Channel conductance substates

Introduction

The potassium inward rectifying currents play an important role in the myocardial cell membrane in the stabilization of the cell resting potential (for a review see Noble 1979). Several papers have recently been published describing the behaviour of single inward rectifying K^+ channels in myocardial cells of guinea-pig (Sakmann and Trube 1984a, b), rabbit (Kameyama et al. 1983) and frog (Momose et al. 1983). These channels had a comparatively large conductance at potentials more negative than the potassium equilibrium potential (V_k) however, at potentials more possitive than V_k (i.e. in physiological conditions) the single-channel currents could not be recorded reliably although a clear integral outward current through

these channels was revealed in the whole-cell configuration (Sakmann and Trube 1984a).

In the present paper two types of inward rectifying K^+ channels are described coexisting in rat ventricular cell membrane. The characteristics of these channels differ from those described earlier (Sakmann and Trube 1984a, b; Kameyama et al. 1983; Momose et al. 1983). One of these K^+ channels types has a detectable conductance at potentials more positive than V_K, and at potentials below V_K, a multitude of conductance substates can occur.

Materials and Methods

Preparation. Rat ventricular cells were isolated using a procedure similar to that described by Wittenberg and Robinson (1981). The heart was perfused through the aorta with nominally Ca^{2+}-free solution containing collagenase (1 mg/ml; Sigma type 1) for 25 min at 27 °C. The ventricles were then chopped into thick slices and placed in 5 ml perfusate containing 300 μmol/l $CaCl_2$, 1.5 mg/ml hyalouronidase (Reanal) and 0.1 % bovine albumin (Sigma, fraction V). Cell suspension was obtained by gentle shaking of the ventricular slices.

Solutions. The normal Tyrode solution contained (in mmol/l): NaCl 120; KCl 5.8; $NaHCO_3$ 4.3; KH_2PO_4 1.4; $MgCl_2$ 1.2; HEPES 5; glucose 11; pH 7.2. The bath solution contained (in mmol/l): NaCl 135; KCl 5.4; $MgCl_2$ 1.2; $CaCl_2$ 0.5; glucose 11; HEPES 5; pH 7.2. The pipette solution contained (in mmol/l): KCl 140; $MgCl_2$ 1.2; $CaCl_2$ 1; HEPES 5; pH 7.2. In some experiments KCl in the pipette was replaced by an equimolar amount of NaCl.

Patch current recording. Ventricular cells dispersed in a recording chamber on the stage of an inverted microscope were prefused with the bath solution. Experiments were performed at room temperature (20—22 °C). The patch-clamp technique was used to record single-channel currents in the cell-attached configuration (Hamill et al. 1981). A home-made current amplifier was used with input dual FET 2SK18A (Toshiba) and a 15 GΩ feedback resistor. Single-channel currents were displayed on a storage-type oscilloscope and stored on magnetic tape. Recordings were treated by computer at 4 ms sampling intervals.

In some experiments the resting membrane potential of ventricular cells was determined following the single-channel current measurement. For this purpose the membrane patch was destroyed. The average resting potential determined from 12 cells was -69 ± 3.8 mV (mean ± S.D.). To obtain the value of the patch membrane potential during the experiment, the difference between the pipette potential and the resting potential measured at the end of the experiment was calculated (absolute patch membrane potential). If this measurement could not be done, the membrane potential was determined with respect to the resting potential of the cell.

Results

The activity of single channels could be recorded in almost all our experiments, original records of currents through them are shown in Fig. 1*A*. We shall term them "type I channels". The activity of other channels was observed more rarely, in about one third of the experiments (Fig. 1*B* type II channels). Currents through both channel types were observed in the cell-attached configuration only. When

the pipette was removed from the cell, forming an isolated inside-out or outside-out patch, single-channel currents observed so far disappeared for several seconds. In the potential range more negative than the reversal potential (V_r) both channel types exhibited linear I—V relation (Fig. 2). In this case, for 140 mmol/l KCl in the pipette, the conductance was 38.9 ± 0.4 pS (mean $\pm$ S.E., $n = 16$) for the type I channels and 25.7 ± 0.7 pS ($n = 14$) for type II channels. A general property of both channel types was a strong inward going rectification. However, while currents through type I channels could not be reliably detected at potentials more positive than V_r, under these conditions outward currents through type II channels could be recorded (see Fig. 1*B*, records at potentials +70 mV and +90 mV). To study the selectivity of channels for K^+ ions a number of experiments was carried

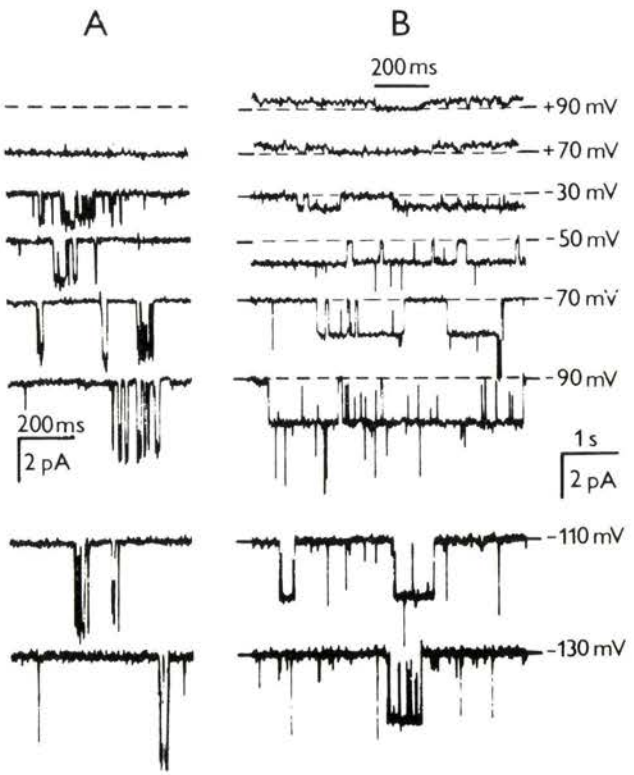

Fig. 1. Currents through inward rectifying K^+ channels of type I (*A*) and type II (*B*) recorded in the same experiment. The patch membrane potentials (absolute values) given at the right of the picture are common for *A* and *B*. Inward currents are downward. Filtering at 1 kHz (−40 dB/dec). Time calibration indicated above the uppermost trace in panel *B* refers to the records at potentials +70 mV and +90 mV. Dashed lines indicate zero-current level. All records were obtained under steady-state conditions.

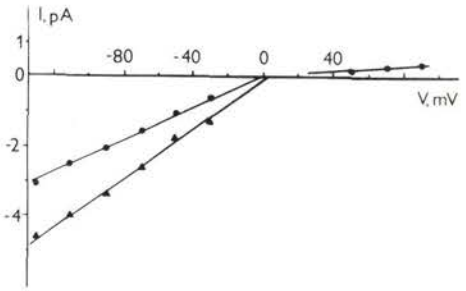

Fig. 2. Single-channel I—V relations from the experiment shown in Fig. 1 (▲) type I channels; (●) type II channels; the patch pipette was filled with 140 mmol/l KCl. The slope conductance of type I channels was 36.9 pS and that of type II channels 25.7 pS at potentials negative to V_k and 4pS at potentials positive to V_K. Abscissa: absolute patch membrane potentials.

out with different K^+ concentrations in the pipette, measuring the cell resting potential (see Methods). As can be seen from Fig. 3*A*, the conductance of both types channels decreased upon decreasing $[K^+]_o$. In this case, zero-current potentials for both types of channels were close to the Nernst potential for potassium (Fig. 3*B*), a concentration of K^+ in the myoplasm of 140 mmol/l (Walker and Brown 1977). These results suggest that in both types of channels the current is mainly carried by K^+.

The kinetic properties of both types of channels strongly differed from each other. The channels of type I were characterized by burst-like activity, while type II

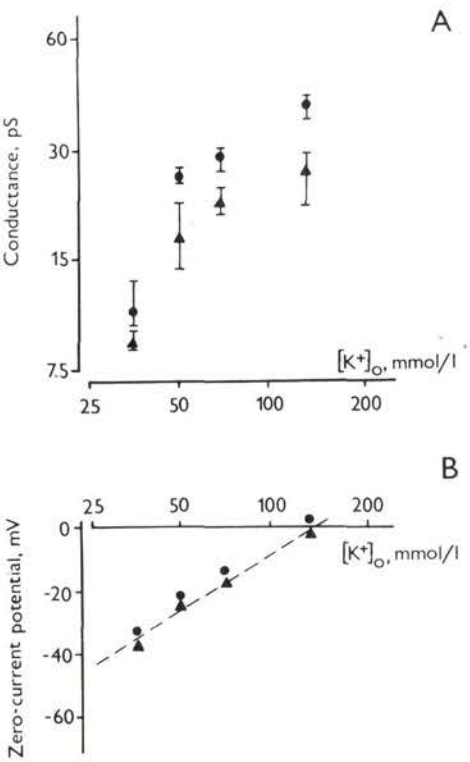

Fig. 3.A: Single-channel conductance as a function of $[K^+]_o$ in double-logarithmic coordinates. (●) type I channels; (▲) type II channels. *B:* zero-current potentials extrapolated from the I—V relations as a function of $[K^+]_o$. A straight line is drawn according to the K^+ equilibrium potentials calculated from the Nernst equation assuming a concentration of K^+ in the myoplasm of 140 mmol/l.

channels behaved in this way much more rarely. In addition, the lifetime of type I channels was much shorter than that of type II channels. The mean open time for type I channels was 18 ms at a membrane potential of -60 mV and 12 ms at -100 mV (the calculations were carried out for gap durations ≥ 4 ms). Unfortunately, the limitations in the computer technique available (too a large sampling interval of ADC) did not allow a comprehensive analysis of the fast kinetics of type I channels. Histograms for gap durations and open times of type II channels plotted for membrane potentials -60 mV and -105 mV are shown in Fig. 4. The mean open time of channels decreased as the hyperpolarization increased (1.3 s at -60 mV and 0.94 s at -105 mV). At the same time, the average gap duration significantly increased with the increasing hyperpolarization (1.85 s at -60 mV and 7.7 s at -105 mV), i.e. the probability of opening decreased. This considerably complicated the statistical treatment taking into account the limited lifetime of the patch. Thus, the "nonsmooth" pattern of the histogram in Fig. 4*d* is explained by

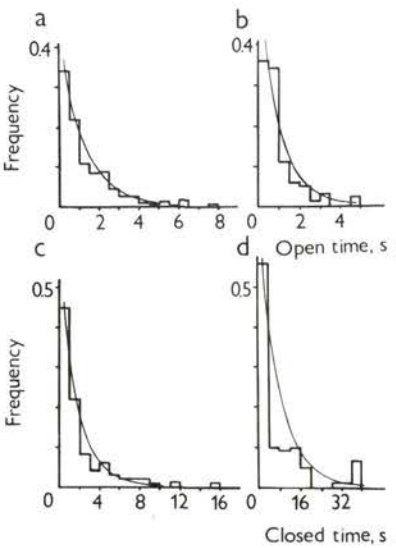

Fig. 4. Histograms of open time (*a*, *b*) and gap duration (*c*, *d*) of type II channels. The histograms were approximately fitted by exponentials; *a*: V (patch membrane potential) $= -60$ mV; τ (time constant of exponentials) $= 1.3$ s; *b*: V $= -105$ mV; $\tau = 0.94$ s; *c*: V $= -60$ mV; $\tau = 1.85$ s; *d*: V $= -105$ mV; $\tau = 7.7$ s.

the fact that at a given potential (-105 mV), only 62 openings were recorded during 15 min of recording time. Voltage dependence of the probability of open-state of type II channels could also be observed when the membrane potential was switched from one level to another. Fig. 5*a*, *b*, *c* shows records from such an experiment with one channel present in the patch. Hyperpolarizing potential jumps were applied from different holding potentials 0 mV (*a*), +25 mV (*b*), +50 mV (*c*) to the cell resting potential. It can be noted that the channel opens immediately or soon after potential jumps, indicating a high probability of open-state at potentials more positive than V_K. This was more pronounced in a similar experiment with several channels present in the patch (Fig. 5*d*). The

probability of channels opening was high at the begining of the negative test pulse, and it decreased to a steady-state value for several seconds and remained unchanged over 15 min of following hyperpolarization.

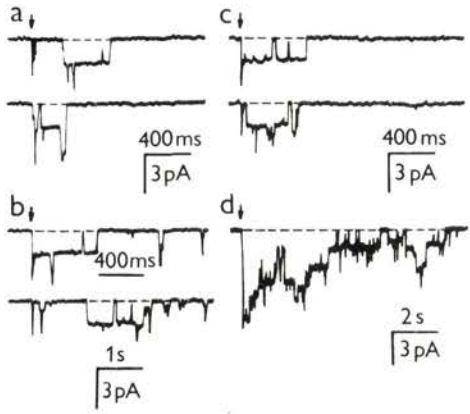

Fig. 5. Type II channels have a high probability of opening at potentials positive to V_K. Negative potential jumps were applied from holding potentials (absolute values): 0 mV (*a*), +25 mV (*b*, *d*), +50 mV (*c*) to the resting potential of the cell (-70 mV); *a*, *b*, *c*: records of single-channel currents obtained under two successive potential jumps (one channel was present in the patch); *d*: another experiment with several channels present in the patch. Dashed lines indicate zero-current levels. The moment of potential switching on is indicated by arrow.

An interesting property of type II channels was the occurrence of conductance substates. This phenomenon was observed in almost all experiments. In most experiments a major conductance state dominated, the others being recorded occasionally. However, in some cases a number of different current sublevels were seen in addition to the main level. An example of an original record from an experiment, in which approximately 3 distinct conductance states of the channel predominated, is shown in Fig. 6*A*. It can be seen that all the conductance states were longlasting and occurred either within one opening or as individual events. The selectivities of different conductance substates of the channel for K^+ were the same as those of the major state. The I—V relations for 3 different conductance states (6.5pS, 14.pS and 21.8pS, respectively) are shown in Fig. 6*B*. Much more channel conductance substates were clearly seen in one of the experiments during 11 min recording time at the resting potential of the cell (Fig. 6*C*). The amplitude of the current through the lowest conductance state of the channel recorded was about 0.1 pA, i.e. near the resolution limit of our amplifier. The next conductance state corresponding to a current amplitude of 0.2 pA was therefore taken as the elementary conductance state. In 262 channel openings the following divisible conductance states could be distinguished (in pS): 2.6 (relative frequency P = 0.12); 5.2 (P = 0.29); 7.8 (P = 0.06); 10.4 (P = 0.015); 13 (P = 0.06); 15.6 (P = 0.08); 18.2 (P = 0.08); 20.8 (P = 0.07); 23.4 (P = 0.09); 26 (P = 0.023); 28.6 (P = 0.01); 31.2 (P = 0.026); 33.8 (P = 0.026); 36.4 (P = 0.01); 39 (P = 0.026); 46.8 (P = 0.004). The maximal conductance measured (46.8 pS) was about twice the mean value (25.7 pS) for the type II channels; it can therefore be assumed that there were two channels in the patch. It should be noted that the average gap

duration in this experiment was 0.42 ms, i.e. essentially less than that during the major conductance level.

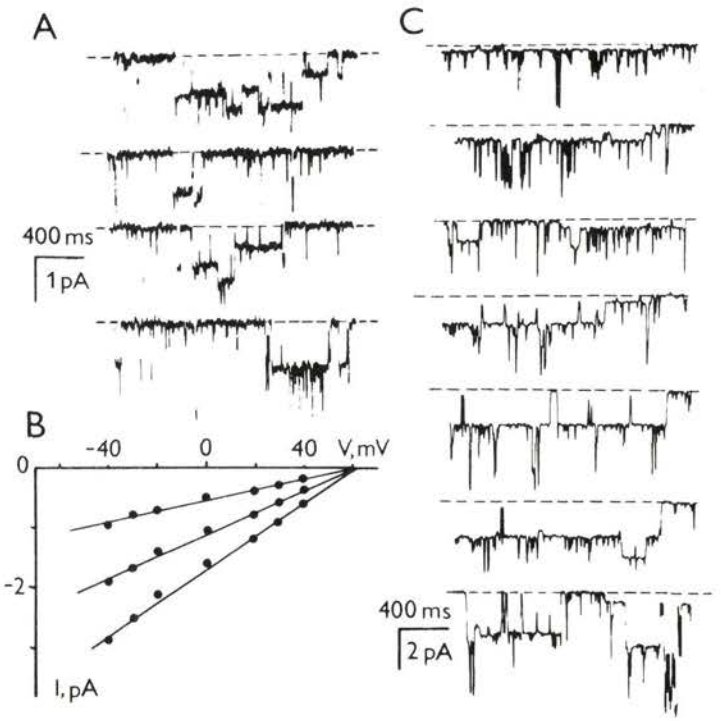

Fig. 6. Different current sublevels of type II channels. Current fluctuations of short durations correspond to the activity of other type of channels present in the patches. *A:* Example of current traces where the patch current oscillates between at least three different current levels. The experiment was performed with 70 mmol/l KCl in the pipette. The patch membrane potential was −20 mV with respect to the resting potential of the cell. The zero current level is indicated by the dashed line. Filtering at 1 kHz (−40 dB/dec). *B:* current-voltage relationships for three current sublevels. The slope conductances given by three different lines were 6.5 pS, 14.3 pS and 21.8 pS. The potential values are given with respect to the resting potential of the cell. *C:* other experiment. An example of records with numerous channel current sublevels. All the records were obtained at the resting potential of the cell (−72 mV). The pipette was filled with 140 mmol/l KCl. Filtering at 100 Hz (−20 dB/dec).

Discussion

Of the two types of inward rectifying K^+ channels in the membrane of rat myocardial cells, described in the present paper, the type I channels are very similar (in their conductance and kinetics) to "resting" K^+ channels in the membrane of rabbit sinoatrial node cells, described by Sakmann et al. (1983). The conductance of type II channels below the reversal potential is approximately the same as that of

inward rectifying K^+ channels in the membrane of guinea-pig heart ventricular cells, described in detail by Sakmann and Trube (1984a). However, the kinetic properties of these later channels are different. The main differences of type II channels as compared with inward rectifying K^+ channels in other myocardial cells include a high activity and detectable conductance (4pS) at potentials more positive than the reversal potential. It is unlikely that outward single-channel currents recorded by Sakmann and Trube (1984a, see their Fig. 1*B*) were currents through the inward rectifying channels because of the low opening frequency of the current steps in the experiments of above authors. While the results of Sakmann and Trube (1984b, see their Fig. 1*A*) and our results (see Fig. 5) have shown that the probability of inward rectifying channels opening at potentials positive to V_k is high. An attempt to record outward currents through the K^+ inward rectifying channels in rabbit ventricular cells has also been undertaking by Kameyama et al. (1983). However, these authors could not record outward single-channel currents under usual conditions (normal Tyrode bath solution and pipette solution containing 140 mmol/l K^+) because of excess noise. These current could be recorded only after the cells were bathed in high K^+ solution (140 mmol/l K^+ with 1 mmol/l EGTA and with 50 mmol/l K^+ in the pipette. The conductance of the channels at potentials positive to V_K was 14 pS; this seems high enough for the channel currents to be detected under usual conditions. Hence, the recorded current steps either corresponded to K^+ channels of another nature, or the treatment of the cell membrane with Ca^{2+}-free, EGTA solution modified the mechanism of rectification.

It has been reported earlier, that in some types of ionic channels there were different conductance substates in addition to the main conductance state (Coronado and Latorre 1982; Geletyuk and Kazachenko 1983; Hamill et al. 1983; Sachs 1983). In particular, this was the case for inward rectifying K^+ channels in guinea-pig myocardial cells (Sakmann and Trube 1984a) where 4 conductance substates were found. Two of them appeared with a low probability and had a short lifetime (a few miliseconds). We have found that the type II channels can adopt a large number of conductance states, with long lifetimes, and that they can be recorded separately. Following arguments can be considered to exclude the explanation of this phenomenon as being an artifact.

1. In our experiments seals values varied from 20 to 90 GΩ (45 GΩ in the experiment shown in Fig. 6*C*). Consequently, the smaller current step-size values can hardly be explained by "rim channels" (Hamill et al. 1981).
2. The existence of different conductance substates in type II channels was observed in almost all experiments; in most cases however, the patch currents oscillated between the main level and different current sublevels. Separate smaller current steps could only rarely be recorded.
3. It might be assumed that channels of another nature with low conductance

were present in the patch under study; the pattern of multiple conductance states might then be explained by superpositions of currents through these channels (see, however statement 2). If this was the case, the density of these channels in the membrane would be very high. For instance, in experiment shown in Fig. 6*C*, the number of such channels in the patch should be more than 20. Thus, currents through these channels would virtually be recorded in all experiments.

Similar data have been presented recently by Geletyuk and Kazachenko (1983), who reported the existence of 16 conductance states for K^+ channels in the snail neuronal membrane. These authors have suggested that the K^+ channel represents a cluster of 16 subunits operating either all together or independently. Our statistical data have been insufficient to provide strong evidences in favour or against this hypothesis.

We could not determine the exact number of conductance states of the channel (about 10 or more) since states may have been present which could not be detected reliably by our electronics.

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