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Single voltage-dependent and outward rectifying K^+-channels in isolated rat heart cells

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Abstract. Studies on single K^+-channel currents recorded from isolated rat heart muscle cells, in which early repolarization is known to be exceptionally fast, are reported here. A K^+-channel which is blocked by TEA (tetraethylammonium) from the inside only has been found.

The total open time of the channel, measured in steady-state after activation, indicated outward rectifying properties. The single channel conductance increases with depolarization from 25 pS at −70 mV to 75 pS at + 70 mV.

Selectivity of the channel has also been measured and it was found that only Rb^+ and K^+ can permeate the channel, whereas the permeability (P) for Li^+, Na^+, Cl^-, Mg^{2+}, and Ca^{2+} is less than 0.05 times P_{K^+}.

Ba^{2+} and Cs^+ block the channel activity.

These results clearly demonstrate the existence of K^+-selective outward rectifying conductance pathways in rat ventricular myocytes.

Key words: K^+-channels, patch-clamp, heart

Introduction

Several types of K^+-channels contribute to excitable membrane functions. In nerve axon, repolarization is governed by a K^+-current with voltage and time dependent activation and outward rectifying properties. To date, little information exists about this delayed rectifier on the single channel level (Conti and Neher 1980). This fast delayed rectifying K^+-current seems to be missing in the mammalian ventricular myocardium, which shows a slow, plateau-type repolarization. Outward movement of K^+ through Ca^{2+}-channels has also been studied in ventricular fibres and may contribute to cellular K^+-efflux (Lee and Tsien 1982).

Here we describe studies on single K^+-channel currents recorded from isolated rat heart muscle cells, in which early repolarization is known to be exceptionally fast (Watanabe et al. 1983).

Our results demonstrate the existence of K^+-selective outward rectifying conductance pathways in rat ventricular myocytes, not so far found in mammalian myocardium.

Materials and methods

Rat ventricular cells were isolated using collagenase treatment (Piper et al. 1982). Between 1 and 6 h after isolation of the cells, single channel currents were recorded with the patch clamp technique (Hamill et al. 1981). Studies were performed at room temperature on excised membrane patches of either orientation, that is, either inside out or outside out (Hamill and Sakman 1980). Fire polished glass pipettes with resistance values between 3 and 10 MΩ were used for gigaseal formation and seal resistance was consistently found to be about 10 GΩ. The number of channels active in the patch was inversely related to pipette resistance and single channels were active throughout the course of experiments which lasted from 20 to 90 min. Single channel recordings were low pass filtered at 0.3 or 1 kHz and stored on PCM tape for further processing. Unless otherwise stated all electrolyte solutions contained 0.5 mM $CaCl_2$ for effective gigaseal formation.

Results

When isolated membrane patches, in symmetrical KCl, were produced, we were able to measure opening and closing of single K^+-channels in almost every patch, resulting in inward or outward current depending on membrane potential. In a given patch, 1–10 channels could be detected, this number being correlated with pipette conductance. Most channels

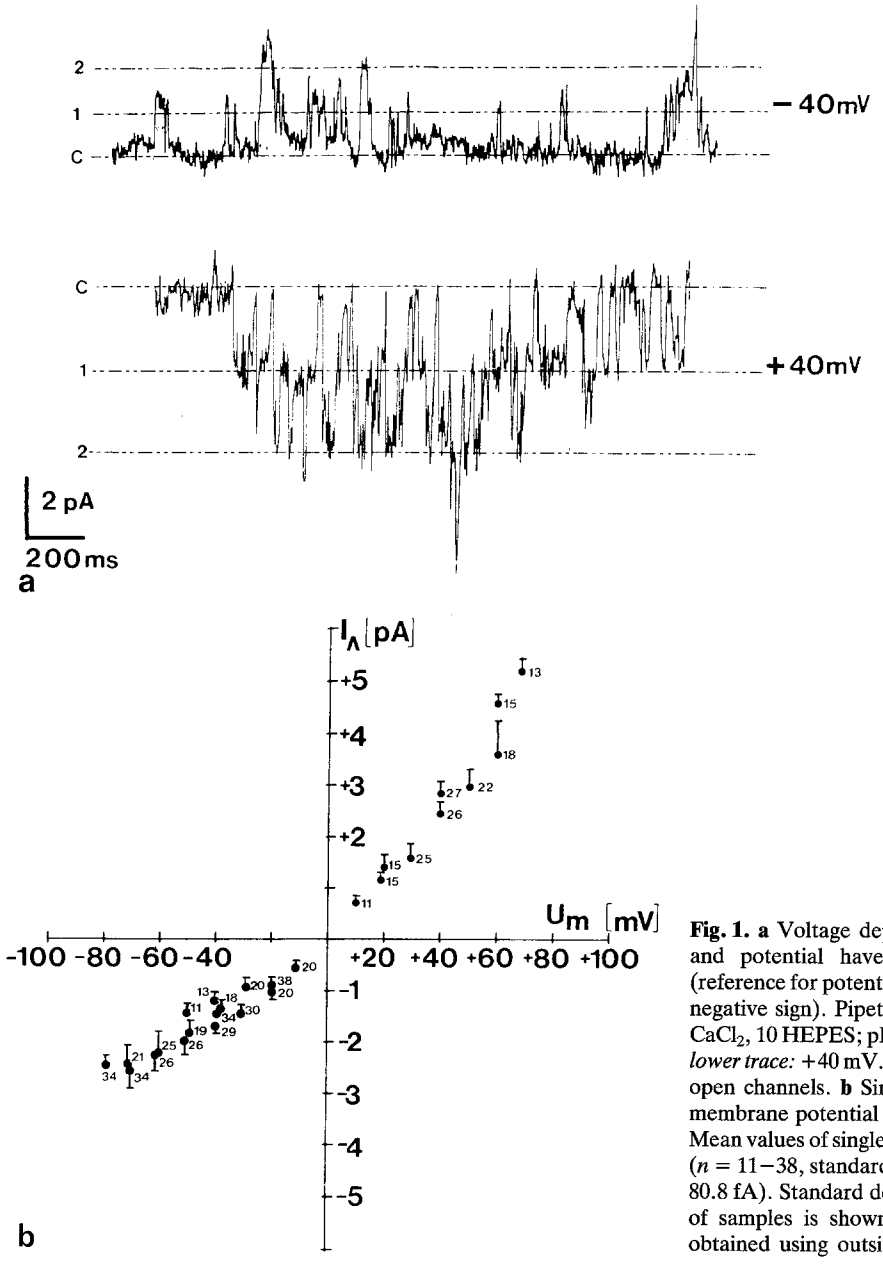

Fig. 1. a Voltage dependence of single channel current. Current and potential have their usual electrophysiological meaning (reference for potential is membrane outside, inward currents have negative sign). Pipette solution and medium (mM): 120 KCl, 0.5 $CaCl_2$, 10 HEPES; pH 7.0. Inside out patch. *Upper trace:* −40 mV, *lower trace:* +40 mV. c indicates closed state, 1 and 2 the number of open channels. **b** Single channel current (I_{lambda}) plotted against membrane potential (U_m). Same experimental conditions as in **a**. Mean values of single channel currents from four inside out patches (n = 11−38, standard error of mean value ranged between 8.9 and 80.8 fA). Standard deviations are shown in the figure, the number of samples is shown beneath each point. Similar results were obtained using outside out patches

that have been studied showed the characteristics described below. Under symmetric ionic conditions the current stepsize for opening and closing events was measured. The conductance of the channel ranged from 25 to 75 pS, depending on membrane potential. The voltage dependence of the single channel current is shown in Fig. 1a. The non-linear dependence of current (I) upon voltage (V) for the single channel resulted in outward rectification of single channel conductance (Fig. 1b). The regression of the I/V relationship was tested for linearity by variance-analysis (Kreyszig 1979) and we calculated a significance for non-linearity better than 0.99. Voltage dependence of channel activity is shown in Fig. 2a. The time delay in activation of the K^+-current is demonstrated in Fig. 2b for a voltage step from −70 mV to −10 mV. The trace represents the average of 125 single channel recordings. Within about 80 ms the current has reached 63% of its maximal value. The average number of open channels at a given voltage has been evaluated; the result is shown in Fig. 2c.

The ion-selectivity of the channel was estimated by measuring the reversal potential of single channel current for different electrolyte compositions (Table 1). Except when Rb^+ was present, the reversal potential always followed the K^+ equilibrium potential. The permeability (P) for Na^+, Li^+, Mg^{2+},

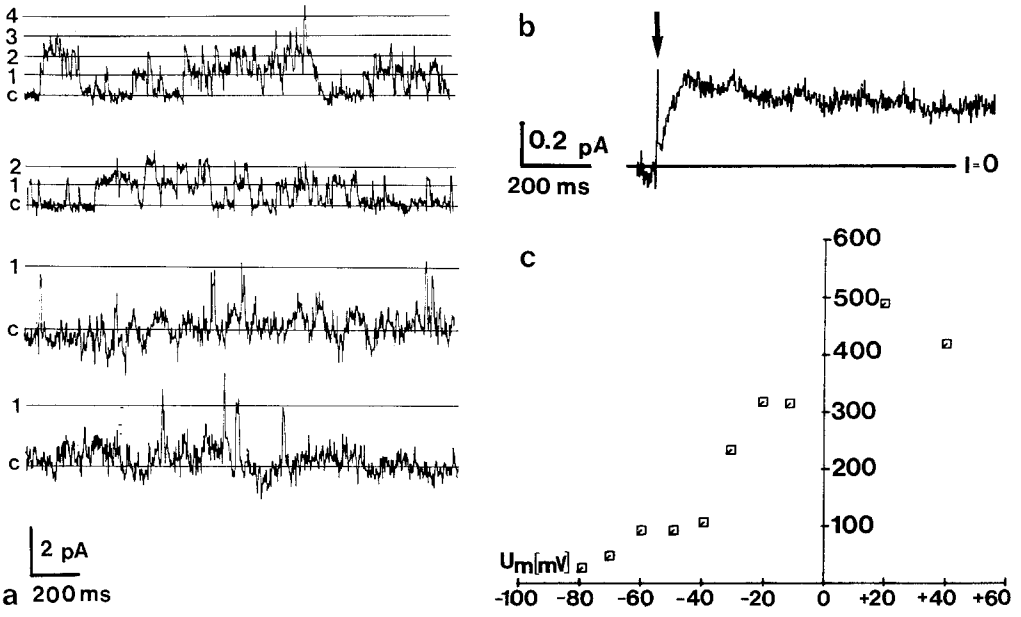

Fig. 2. a Patch currents at different membrane potentials. Pipette solution and Medium (mM): 120 KCl, 0.5 $CaCl_2$, 10 HEPES; pH 7.0. Inside out patch. *Upper two traces:* −20 mV; *lower two traces:* −70 mV. *c* indicates the closed state, 1−4 the number of open channels. **b** Average current of 125 single channel recordings, showing activation of the K^+-current after a voltage jump from −70 mV to −10 mV (indicated by *arrow*). Capacitance and background currents are compensated. Pipette solution (mM): 120 KCl, 0.5 $CaCl_2$, 10 HEPES; pH 7.0. Medium (mM): 116 NaCl, 7 KCl, 0.8 $MgCl_2$, 0.5 $CaCl_2$, 10 HEPES; pH 7.0. Outside out patch. **c** Relative number of open channels at a given membrane potential. For each voltage, record length was 8 s and in each 10-ms interval the number of open channels was counted. As the actual number of channels in the patch area is not known, total open time is expressed in arbitrary units (100 counts/division). Pipette solution and medium (mM): 120 KCl, 0.5 $CaCl_2$, 10 HEPES; pH 7.0. Inside out patch

Table 1. Selectivity of the channel. Cation concentrations of medium and pipette are expressed in millimole. The measured reversal potential U_{REV} (MEAS) is displayed. U_{REV}(IDEAL) is the reversal potential calculated for a K^+-channel with ideal selectivity. The permeability ratio P_{Cation}/P_{K+} was calculated according to the Goldman equation (Goldman 1943). Parallel to the estimation of P_{Na+}, a second set of electrolyte compositions was taken to demonstrate that the reversal potential follows the K^+-equilibrium potential. For the determination of $P_{Ca\,2+}$, symmetrical Ca^{2+} concentrations were taken to demonstrate the same behaviour

ELECTROLYTE COMPOSITION			$U_{REV.}$(MEAS.)	$U_{REV.}$(IDEAL)	P_{CATION}/P_{K^+}	
	MEDIUM	PIPETTE				
Na^+	135	20	−67 mV	−75 mV	0,02	P_{Na^+}
K^+	6	122				
Na^+	18	″	+2 mV	−2 mV	/	
K^+	95	102				
Ca^{++}	0,5	0,5				
Na^+	123	20	−10 mV	−80 mV	1,67	P_{Rb^+}
K^+	5	122				
Rb^+	45	″				
Na^+	129	20	−76 mV	−78 mV	0,04	P_{Li^+}
K^+	5	122				
Li^+	48	″				
Na^+	129	20	−75 mV	−86 mV	0,04	$P_{Mg^{++}}$
K^+	4	122				
Mg^{++}	49	″				
Na^+	129	20	−77 mV	−86 mV	0,03	$P_{Ca^{++}}$
K^+	4	122				
Ca^{++}	48	0,5				
Na^+	110	″	−80 mV	−79 mV	/	
K^+	4	102				
Ca^{++}	0,5	0,5				

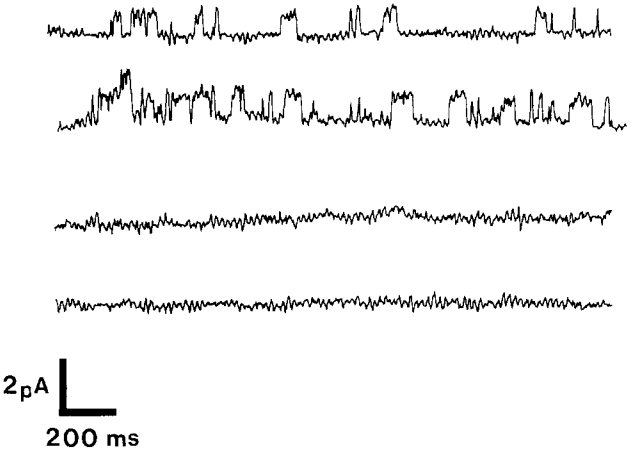

Fig. 3. Block of single channel activity by TEA. Pipette solution (mM): 120 KCl, 20 NaCl, 0.5 $CaCl_2$, 10 HEPES; pH 7.0. Medium (mM): 116 NaCl, 7 KCl, 0.8 $MgCl_2$, 0.5 $CaCl_2$, 10 HEPES; pH 7.0. Membrane potential: 0 mV. Inside out patch. With the given electrolyte composition the channel openings reflect inward directed membrane currents. *Upper two traces:* before addition of TEA, *lower two traces:* after addition of TEA (50 mM) to the medium facing the inner side of the membrane

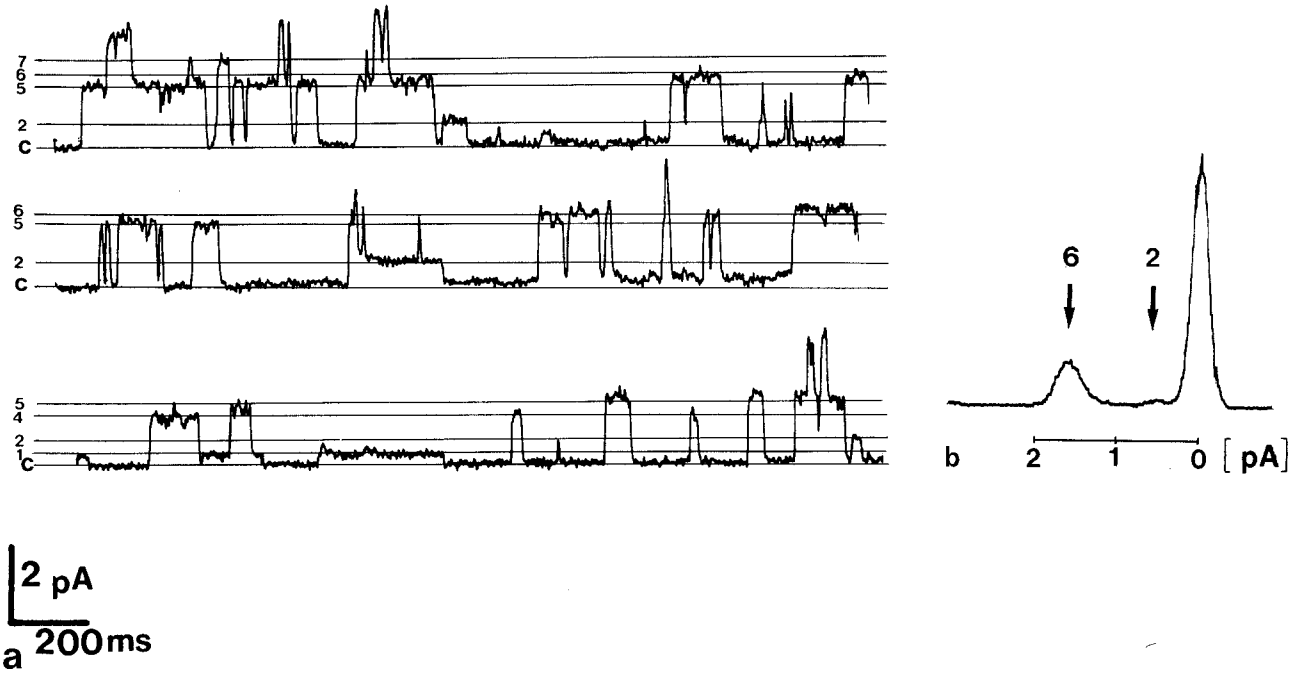

Fig. 4. a Single channel recordings indicating stepwise changes in channel current. Pipette solution (mM): 122.4 KCl, 10 HEPES; pH 7.0. Medium (mM): 135 NaCl, 1.2 KCl, 2 $CaCl_2$ and 10 HEPES; pH 7.0. Outside-out patch. Detected sublevels of conductance are marked with numbers from 1 to 7 with increasing single channel current. Membrane potential = 0 mV. **b** Ampliude histogram of single channel currents, showing the representation of sublevels. Same experimental conditions as in **a**, record length 60 s. The sublevels No. 2 and 6 are indicated by *arrows*

Ca^{2+}, and Cl^- was calculated to be less than 0.05 times P_{K^+}, whereas P_{Rb^+} was about 1.7 times P_{K^+}.

To further characterize the channel we used several blocking agents, known to block K^+-currents in other preparations. When TEA (tetraethylammonium$^+$, 50 mM) was applied to the inside of the membrane, single channel currents disappeared (Fig. 3), whereas TEA applied to the outside showed no effect. Cs^+ (50 mM), applied to the outside of the membrane, blocked single channel currents for the inward, but not for the outward, direction. Ba^{2+} (50 mM) also blocked single channel activity, when applied to the outside. Absence of Ca^{2+}, on the inside and/or on the outside of the membrane did not change single channel activity. The drug, D600, was ineffective in blocking single channel current when applied to the outside of the membrane at a concentration of 5 μM, a concentration commonly used to block Ca^{2+} currents in this and other preparations (Lee and Tsien 1982).

In outside out patches under conditions similar to physiological ionic conditions (122.4 mM KCl, 10 mM Hepes (pH: 7.0) in the pipette and 135 mM NaCl, 1.2 mM KCl, 2 mM $CaCl_2$ and 10 mM Hepes (pH: 7.0) in the medium), the membrane noise is reduced, but the current measured through a single channel at a given voltage does not always jump between only two levels ("open" and "closed"), but exhibits other discrete sublevels (Fig. 4a). Figure 4b shows the amplitude histogram of a single channel trace. The distribution of sublevels for this particular record is indicated.

Discussion

The voltage dependence, single channel conductance, selectivity, and blocking properties of the channel characterized here show, that it differs considerably from other K^+-channels so far described in cardiac tissue. The effects of several blocking agents of K^+-currents were tested. The side-specific action of TEA supports the view of an outward rectifying K^+-channel. Similar results have been obtained with delayed rectifier K^+-channels from other tissues and species, whereas inward rectifying channels have been found to exhibit an external blocking site (for review see Latorre and Miller 1983). Inward rectifying K^+-selective channels, which probably determine resting potential, have also been found in ventricular myocytes, but in contrast to the channels we have measured they did not show any outward currents (Trube and Hescheler 1983).

The unidirectional blocking of K^+-current by Cs^+ is in good agreement with data from delayed outward currents obtained using voltage clamp methods on several preparations (Dubois 1983).

Outward K^+-current flow through non-K^+-selective Ca^{2+}-channels has been observed (Lee and Tsien 1982), but under physiological ionic conditions these currents occur at potentials beyond +50 mV. These K^+-currents through Ca^{2+}-channels of guinea pig heart cells cannot be blocked by addition of internal TEA (Lee and Tsien 1982). Additionally we have tested the drug D600 (5 μM), which is known to block Ca^{2+}-currents (Lee and Tsien 1982) and did not find any effect on single channel activity.

Both for the Ca^{2+}-activated K^+-channel and for the inward rectifying K^+-channel little or no permeability for Rb^+ has been found (Petersen and Maruyama 1984; Stanfield 1983), whereas in our own study Rb^+ permeates and does not block delayed rectification.

The clear non-linearity of the single channel I/V relationship observed under symmetrical ionic conditions might arise for different reasons. (i) Energy barriers with different size at the outer end and at the inner end of the channel can result in rectification of single channel currents. Such models predict exponentially shaped I/V relations (Jack et al. 1975). (ii) Numerous sublevels of single channel conductance under asymmetrical ionic conditions have been found. Voltage dependence of the occurrence of different sublevels under symmetrical ionic conditions (but not resolved in time) could result in a non-linear I/V relationship. (iii) Asymmetric distribution of fixed surface charges can result in different ionic concentrations in the diffuse double layers on both sides of the membrane. This can then result in an asymmetric I/V relationship, even under symmetric ionic conditions in the bulk phases (Jack et al. 1975).

Not only the single channel conductance, but also the average number of open channels increases with depolarization of the patch under the given experimental conditions. When single channel traces were averaged, activation of the outward current was found, the time course being well within the range of repolarization of the action potential of rat ventricular cells (Watanabe et al. 1983).

The time course of the single channel traces shown in Fig. 4a demonstrates the existence of different sublevels of channel conductances. In our experiments under asymmetrical ionic conditions, we have been able to observe all the different conductance states indicated in Fig. 4a, either isolated, that means that the channel remains in only one open state during the open time, or transitions between several open states occurred. Often jumps in patch current from the closed state to states with high conductances are observed; occasional overlap of opening and closing events of different, independent K^+-channels with different conductivities cannot explain this behaviour as these events are too often correlated in time, i.e., they are observed to occur coincidentally within the time resolution of our equipment.

In conclusion, our experiments show, for the first time, the existence of K^+-selective channels with outward rectifying properties in mammalian ventricular myocytes. The major features of this channel are similar to those of channels in tissues like nerve and skeletal muscle (Dubois 1983); it most likely serves as the key-pathway for K^+-outward currents in rat heart cells. Among mammalian ventricular cells, those from the rat show unusual electrophysiological features, i.e., early and fast repolarization. It may be because of this species difference that K^+-selective outward currents from single channels with outward rectifying properties have so far not been detected in mammalian myocytes.

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