On the Apparent Absence of Maxi-K^+ Channel in Rat Aortic Myocyte (43560)

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Abstract. **In cultured rat aortic myocytes, no high-conductance Ca^{2+}- and voltage-dependent K^+ channels (maxi-K^+ channels) such as those found in the human and rabbit were observed. However, we have found that in freshly dissociated rat aortic myocytes, the activities of high-conductance Ca^{2+}- and voltage-dependent channels were present and predominant. In cell-attached patches measured with pipettes filled with normal saline solution, their single channel conductances were 139.6 ± 2.5 picosiemens (mean ± SE). Under the 140-m*M* symmetrical K^+ condition (both bath and pipette solutions contained 140 m*M* KCl), that measurement became 207.2 ± 3.6 picosiemens and increased by about 50% with detaching of the membrane patch. The relative conductances of the channel to K^+, NH_4^+, and Rb^+ were 1:0.61:0.48. The conductance of these channels can readily be reduced by more than 90% by extracellularly applied 0.5 m*M* tetraethylammonium chloride. These characteristics show that they were maxi-K^+ channels.**

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High-conductance Ca^{2+}- and voltage-dependent K^+ channels (maxi-K^+ channels) are widely distributed in many vertebrate-excitable cells. These channels are characterized by large single-channel conductance (100–250 picosiemens [pS] under symmetrical K^+ condition) and blockade by extracellular tetraethylammonium chloride (TEA) at concentrations of less than 1 m*M* (1, 2). In cultured aortic myocytes of both human and rabbit origins, maxi-K^+ channel activity predominates. Under the symmetrical K^+ condition, their single-channel conductances are larger than 200 pS (3, 4). In cultured rat myocytes, however, no such channel activities are recorded. Sadoshima *et al.* (5) observed Ca^{2+}-sensitive K^+ channels with conductance of 135 pS under the symmetrical K^+ condition, but they can only be blocked by TEA at concentrations higher than 10 m*M*. Shoemaker and Worrell (6) found only Ca^{2+}-sensitive K^+ channels with single-channel conductance of 55 pS. Pavenstädt *et al.* (7) reported that K^+ channels are rarely found at all. This work studies the question of the apparent absence of a typical maxi-K^+ channel from rat myocytes.

Materials and Methods

Aortic myocytes were enzymatically loosened from isolated strips of rat aorta with collagenase (0.2%, Sigma Type I) and elastase (0.1%, Sigma Type IIA) at 37°C for 30 min and dispersed by gentle trituration. The dissociated aortic cells were recognized because they contracted with the application of norepinephrine (0.2 μM, in normal saline).

The volume of the experimental chamber was 0.3 ml. In all experiments, bath solution was perfused at a steady rate of 1.2 ml/min. Unless otherwise specified, the bath and pipette solution for the single-channel recordings contained either 140 m*M* KCl, 0.1 m*M* $CaCl_2$, 0.6 m*M* EGTA, and 10 m*M* HEPES (pH 7.2) (K^+ solution; it is called symmetrical K^+ condition when K^+ solution was used both as the bath and the pipette solution), or 130 m*M* NaCl, 5.4 m*M* KCl, 2 m*M* $CaCl_2$, 1 m*M* $MgCl_2$, and 10 m*M* EGTA (pH 7.2) (Na^+ solution). The free calcium ion concentration of the solutions was calculated with the following equation (8).

$$\text{Add Ca} = \frac{1 + K'([\text{EGTA}] + [\text{Ca}])}{1 + [\text{Ca}] \times K'} \times [\text{Ca}] \quad [1]$$

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where [Ca] is the free-Ca^{2+} concentration, [EGTA] is the EGTA concentration, K' is the apparent association constant for Ca-EGTA ($10^{7.1}$ at pH 7.2 and 22°C). The pipette solution used for whole-cell current recording contained 140 m*M* KCl, 0.1 m*M* $CaCl_2$, 0.6 m*M* EGTA, 1.5 m*M* $MgCl_2$, 2 m*M* ATP-potassium salt, and 10 m*M* HEPES (pH 7.2 adjusted with KOH). In order to exclude the flow of K^+ through Ca^{2+} channels, the bath solution used in whole-cell current recording contained 140 m*M* KCl, 0.57 m*M* $CaCl_2$, 0.6 m*M* EGTA, and 10 m*M* HEPES (pH 7.2 adjusted with KOH). This is chosen so that the free Ca^{2+} concentration of the bath solution (10^{-6} *M*) is sufficient to block the flow of K^+ through the Ca^{2+} channels.

A conventional tight-seal method (9) was used. The seal resistance between the patch and the electrode tip was 10–20 gigaohms. For the whole-cell current studies, the signals were stored directly in a PC AT-compatible computer using pCLAMP software (Axon Instruments, Inc.) with the associated analog-to-digital converter. For single-channel activity studies, the signals were recorded on videotape through a 5-kHz filter for subsequent analysis.

The method of data analysis can be found in studies by Brink and Fan (10), Ramanan and Brink (11), and Ramanan *et al.* (12). A histogram of current distribution was generated using point by point analysis. The sampling time was 100 μsec.

The relative permeability of test ion X^+ to K^+ (P_K/P_X) was calculated from the shift of the reversal potential upon changing the bath solution in inside-out detached patch experiments from KCl to equimolar XCl with constant K^+ concentration in the pipette (13, 14):

$$E_x - E_K = \frac{RT}{F} ln \left(\frac{P_K[K^+]_i}{P_X[X^+]_i} \right) \qquad [2]$$

with *R*, *T*, and *F* having their usual meaning.

All experiments were performed at room temperature (20–22°C).

Results and Discussion

In cell-attached patches of freshly dissociated rat aortic myocytes under symmetrical K^+ condition and constant holding potentials, only K^+ channel openings were observed. Three types of K^+ channels with different single-channel conductances could be seen. Their conductances under the symmetrical K^+ condition were (mean ± SE pS) 24.3 ± 1.3 (n = 9; hereafter, 24-pS channel), 66.7 ± 2.7 (n = 8; hereafter, 66-pS channel), and 207.2 ± 3.6-pS (n = 15; hereafter, 207-pS channel). These values were calculated from the slope of i-V curves near zero holding potential. The activity of the 207-pS channels was always predominant. Only a few membrane patches show channels with all three conductance levels. In the 15 patches we examined, 13 of them showed either 207-pS channel with 24-pS channel (n = 8) or 207-pS channel with 66-pS channel (n = 5). The contribution of each K^+ channel in the total current passing through the membrane patch was proportional to the product of the conductance and the open probability of that channel. For those eight patches with 24-pS channel, currents passing through 207-pS channels accounted for 96.5 ± 1.2%. For the other five patches, current passing through 207-pS channels accounted for 94.7 ± 2.1%. There were only two patches in which activities of channels with all three different conductances were seen. Figure 1A shows the current record from a cell-attached patch under the symmetrical K^+ condition. In order to show the three types of K^+ channel in one trace, we intentionally selected the record from a cell with channel openings for all three different conductances. To rule out the possible contribution of Cl^- currents, Cl^- had been substituted by gluconate in five experiments. The results obtained were the same.

The conductance of the 207-pS channel measured with a pipette filled with Na^+ solution and bathed in K^+ solution did not change significantly with patch detachment. The measurements obtained before and after the process were 139.6 ± 2.5 pS (n = 10) and 150.7 ± 3.0 pS (n = 5), respectively. With Student's t test, the P-value for the difference was greater than 0.1. However, under the symmetrical K^+ condition, their single-channel conductances consistently showed considerable increase upon detachment of the patch (Fig. 1B). They increased from 207.2 ± 3.6 pS (n = 15) to 305.9 ± 5.4 pS (n = 9). The difference is statistically highly significant ($P < 0.001$).

The 207-pS channel was highly sensitive to extracellular TEA. In four outside-out detached patches, 0.5 m*M* TEA applied extracellularly reduced the conductance of the channel by more than 90%. In seven inside-out detached patches, 10 m*M* TEA applied intracellularly had no effect.

In cell-attached patches, the open probability of the 207-pS channel showed no significant change as the Ca^{2+}-concentration of the bath solution was changed from 10^{-8} to 10^{-3} *M* (<10%, n = 6, Student's t test, $P > 0.05$). However, their open probability was increased with both the increase in the depolarization level (Fig. 2A; cell-attached patches, n = 6) and the intracellular Ca^{2+} concentration (Fig. 2C; inside-out detached patches, n = 7). Under symmetrical K^+ conditions, as the holding potential increased from 0 to 30 mV, the increase in open probability was due mainly to the decrease in mean closed-time of the channel. From 30 mV on, the increase was due mainly to the increase in mean open-time of the channel (Fig. 2B; n = 6).

To determine whether the 207-pS channel is a K^+-selective channel, the effect of substitution of intracellular K^+ with other monovalent ions was tested. In inside-out detached patches, when K^+ in the bath so-

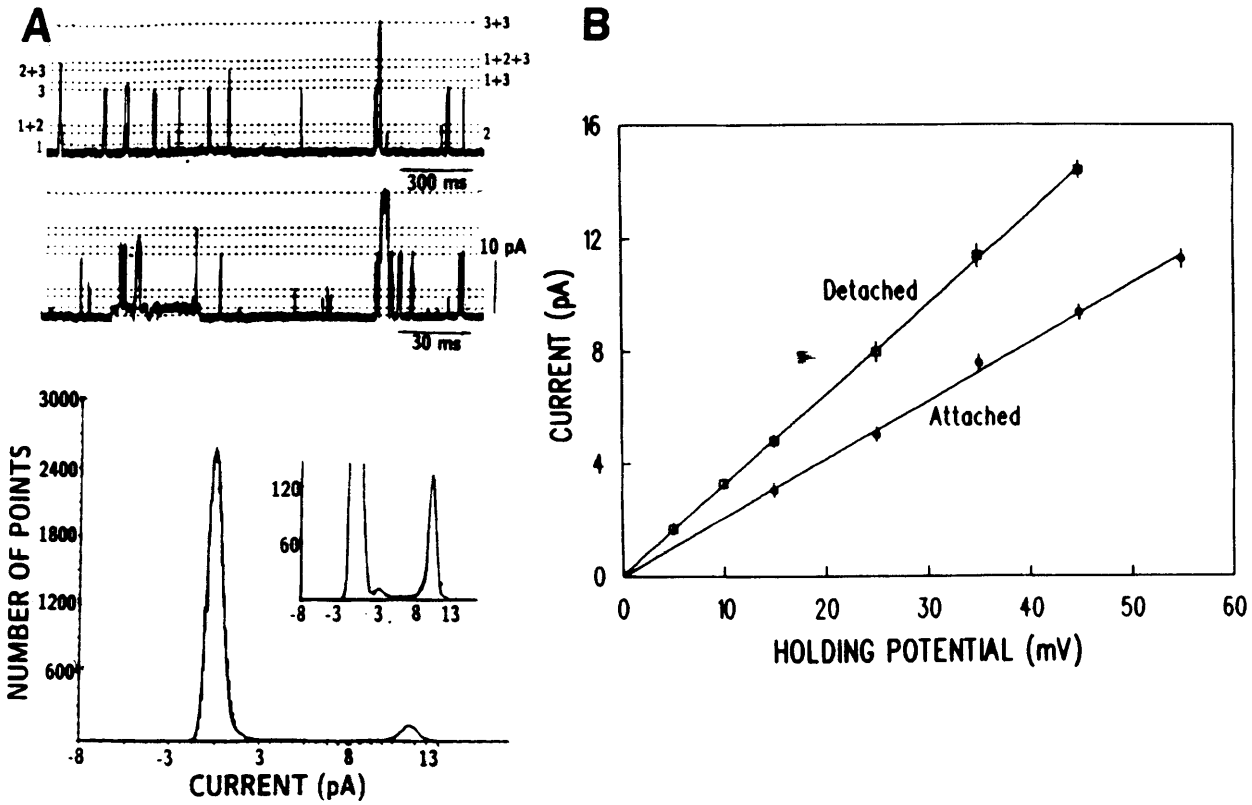

Figure 1. K^+ channel activities from freshly dissociated rat aortic myocyte. (A) Channel activities recorded from a cell-attached patch under the symmetrical K^+ condition. The holding potential was 60 mV. Shown are, from top to bottom, current records at two different sweep speeds and a current distribution histogram. The latter can be fitted under the assumption that there were three active channels with unitary currents of 1.25, 3.35, and 10.95 pA (levels 1, 2, and 3 in current trace) and open-probabilities 0.01, 0.008, and 0.07, respectively. The inset shows the portion of curve with the ordinate expanded. (*B*) i-V curve of channels with largest conductance in membrane patches before (n = 15) and after detachment (n = 9) under the symmetrical K^+ condition (mean ± SE).

lution was totally substituted by Li^+, Cs^+, Na^+, or TEA^+, outward single-channel current flow was not detected, even at holding potentials equal to +70 mV. This was independent of whether the pipette was filled with Na^+ or K^+ solution. When K^+ in the bath solution substituted by Rb^+ or NH_4^+, outward current was readily detected. When measured with a pipette filled with K^+ solution (Fig. 3), the single-channel conductances were 108.1 ± 2.1 pS (n = 6) and 78.5 ± 2.6 (n = 5) for Rb^+ and NH_4^+, respectively. The positive shifts of reversal potential were 18.9 ± 0.6 mV (n = 6) and 12.8 ± 0.4 mV (n = 5) for Rb^+ and NH_4^+, respectively. Using Equation 2 (see Materials and Methods), the relative permeabilities of the 207-pS channel to K^+, Rb^+, and NH_4^+ were: $P_K:P_{NH}:P_{Rb}$ = 1:0.61:0.48. When using a pipette filled with Na^+ solution, the single-channel conductances were 59.8 ± 1.4 pS (n = 4) and 40.0 ± 1.0 pS (n = 5) for Rb^+ and NH_4^+, respectively. The positive shifts of reversal potential were the same as those obtained with the K^+ pipette solution.

The following results obtained show that the 207-pS channels in freshly dissociated rat aortic myocytes are maxi-K^+ channels. (i) The channel was selective to K^+; (ii) the single-channel conductance in cell-detached patches was about 200-pS under the symmetrical K^+ condition; (iii) the open probability was sensitive to both membrane potential and to intracellular Ca^{2+} concentration; (iv) the channels were sensitive to 0.5 m*M* TEA applied extracellularly but insensitive to TEA up to 10 m*M* applied intracellularly. The results also show that the predominant channel activity in freshly dissociated rat aorta myocytes during steady depolarization is that of the maxi-K^+ channel, such as that found in humans (3) and rabbits (4). The fact that the 207-pS channels reported in this work have not been observed previously may be because either the channels were inactive or lost, or their characteristics—such as unit conductance and sensitivity to TEA—changed under the cultural condition. Since the activity of K^+ channels in both Sadoshima *et al.*'s (5) and Shoemaker and Worrell's (6) preparations was quite high, and since in freshly dissociated myocytes only the 207-pS channels have such high activities and the K^+ channel activity almost disappeared after long culturing (7), the second possibility, that the characteristics of maxi-K^+ channels might be changed with culturing, seems to be more plausible.

Rat aortic myocytes were used as the material for

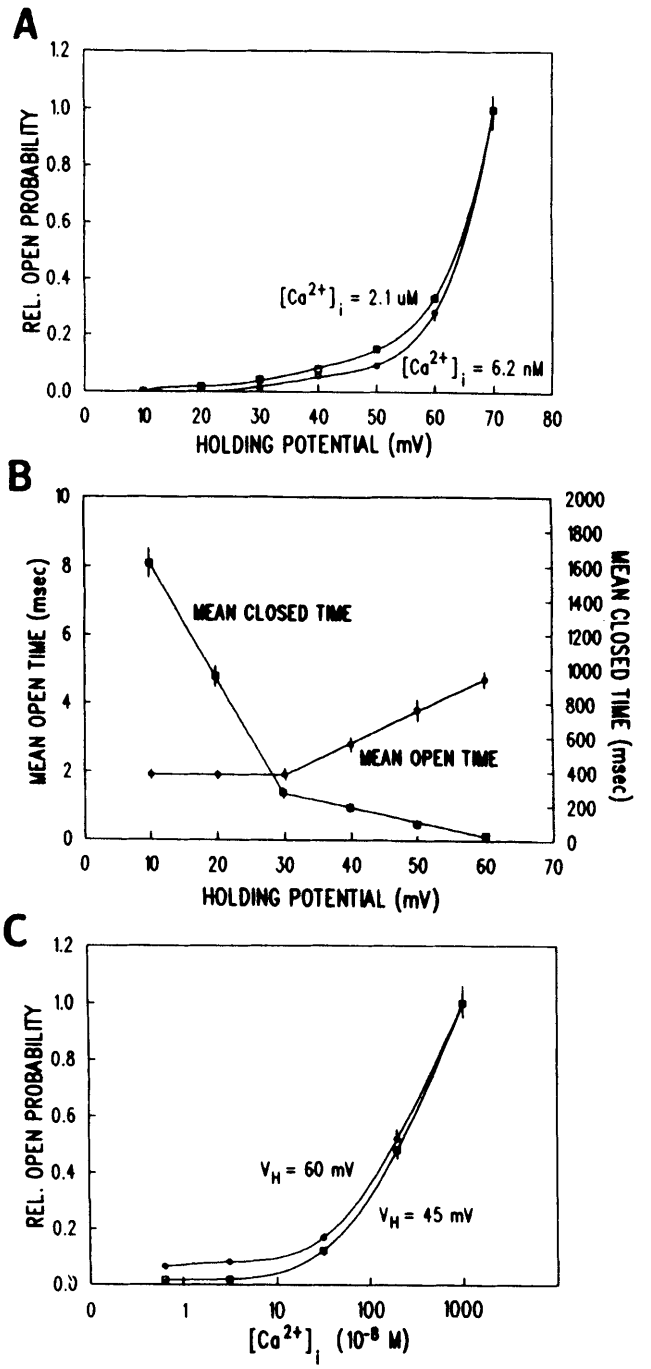

Figure 2. Voltage and $[Ca^{2+}]_i$ dependence of open probability of maxi-K^+ channels in membrane patches under the symmetrical K^+ condition (mean ± SE). (A) Voltage dependence (n = 6). (B) Change of mean open-time and mean closed-time with holding potentials (n = 6). Data shown in (A) and (B) were obtained from cell-attached patches. (C) $[Ca^{2+}]_i$ dependence (n = 7). Results were obtained from inside-out detached patches.

studying the K^+ conductance of the arterial smooth muscle cells. It is important to keep in mind that the results obtained from cultured rat aortic cells may be different from those obtained from the aortic cells of other species.

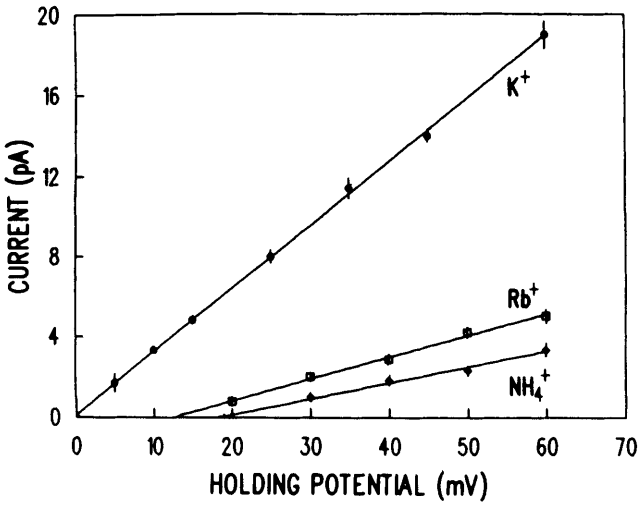

Figure 3. i-V relationships of maxi-K^+ channels in inside-out cell-detached patches bathed in 140 mM K^+ (n = 9), Rb^+ (n = 6), and NH_4^+ (n = 5) solutions (mean ± SE).

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