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ATP-sensitive K^+-channels in the human adult ventricular cardiomyocytes membrane

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Using the patch-clamp method in 27 different inside-out patches it was shown for the first time that they defined the basic parameters of the functioning of single ATP-sensitive K^+ channels in the human adult ventricular cardiomyocytes membrane. At $[K^+]_o$ = 140 mM single channel conductance (over the linear part of the I–V relation) reaches 100 pS. The possibility of the existence of these channels' conductance sublevels as well as the cluster character of their localization in sarcolemma is shown. The channel activity demonstrates an obvious run-down with τ at about a minute The analysed channels possess one open and two closed states.

ATP-sensitive K^+-channel, Human adult ventricular cardiomyocyte

1. INTRODUCTION

The extreme growth of the appearance of a great number of experimental works devoted to ATP-sensitive K^+ channels in the cardiomyocytes membrane [1–9] is to a great degree explained by the desire to find out the molecular mechanisms of potassium permeability change in case of the exhaustion of the ATP storage in them. Though the importance of such research works for experimental and clinical cardiology is difficult to underestimate the peculiarities of single ATP-sensitive K^+ channels in the human adult ventricular cardiomyocytes membrane have not yet been characterized. This research is particularly necessary as the currents through these channels possess some species variations [1,2,5,10].

2 MATERIALS AND METHODS

Individual cardiomyocytes were isolated from myocardium taken from 14 patients at the age of 6–43 years who were undergoing the surgical treatment for the following diseases: interventricular septal defect, mitral valve defect, Fallot's tetrad, etc.

After isolation fragments of ventricular tissue of about 10 × 10 × 10 mm were rinsed in a saturated gas mixture of 95% O_2 and 5% CO_2 at room temperature (21–23°C) in a medium for the preparation of cardiomyocytes (MPC; containing (in mM): NaCl 100, KCl 10, KH_2PO_4 1.2, $MgSO_4$ 5, glucose 20, taurine 30, MOPS 10, pH 7.2) with $CaCl_2$ in the concentration 140 μM and 5% fetal bovine serum. The period of time from the isolation of tissue to the beginning of treatment was not more than 10 min. All the following manipulations during the cell isolation were made at a temperature 37°C

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Tissue was transferred into a fresh portion of the same medium and minced into 2–3 mm pieces with scissors Afterwards the medium was removed and discarded. Tissue was treated initially for 2 min with MPC with EGTA (0.08%) and $CaCl_2$ at a concentration 140 μM. Then tissue was transferred into a specially designed disaggregation vessel [11] containing 4 ml of 0.1% collagenase type A, 0.02% pronase E, 0.1% albumin bovine fraction V and $CaCl_2$ at a concentration of 140 μM in MPC and treated for 10 min. The medium containing isolated myocytes was then centrifuged at 70 × g for 10 min at room temperature. The centrifuged medium was decanted leaving a loose pellet of cells which was gently resuspended in 2 ml of MPC and stored at room temperature. Three times repetition of the procedure helped achieve 60–70% tissue disaggregation The separation of the cardiomyocytes from other cells and debris in the received suspension was conducted by means of sedimentation in the 5% solution of the BSA fraction V in the MPC at 1 g during 10 min at the average room temperature. The separated suspension contained 40–50% cardiomyocytes with the in vivo morphology, which were used in the electrophysiological experiments. The resting potential evaluated in whole-cell patch mode by means of the patch-clamp method [12] was equal to 68 ± 3 mV (mean ± S.D., n = 6) for such cells

Single ion channel records were obtained from excised inside-out membrane patches [12] as has been described before [9]. The pipettes were filled either with MPS with 1 mM $CaCl_2$ or with the K_o solution containing (in mM): KCl 140, MOPS 10, $CaCl_2$ 1, $MgCl_2$ 1, pH 7.2 The bath K_i solution contained (in mM): KCl 140, HCl 70, MOPS 10, $CaCl_2$ 0.3, EGTA 5, pH 7.2. ATP was obtained from Sigma. All experiments were conducted at room temperature (21–23°C).

3. RESULTS

The current through single ATP-sensitive K^+ channels appeared after the transition from the cell-attached mode into the inside-out mode (in 27 out of 136 different membrane patches) with the time delay reaching several seconds. The examination of their sensitivity to ATP (produced with the K_i solution) applied on the inner side of the membrane patch showed that with the

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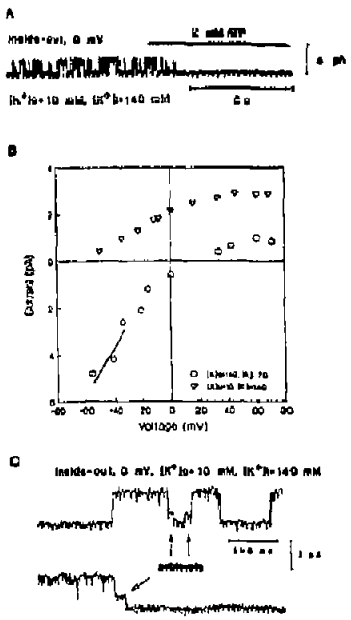

Fig. 1. Properties of ATP-sensitive K^+-channels in the human adult ventricular cardiomyocytes membrane (A) Blocking of the channel by ATP application on the inner side of the membrane patch. The dotted line (here and further) indicates the zero current level, the upward deviation corresponds to outward current. (B) Current–voltage relations, inside-out patch mode. (C) The existence of the single channel conductance sublevels.

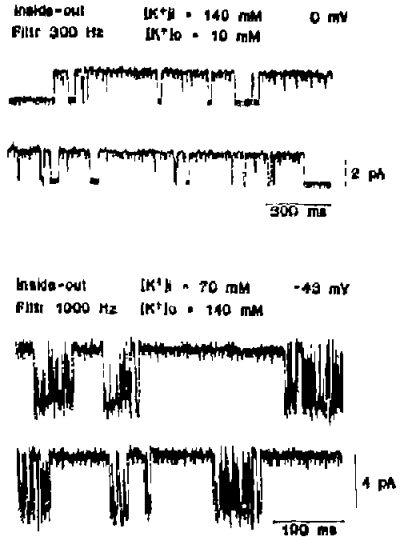

Fig. 2. Current recordings through the single channel. The shown recordings of the outward currents (the 2 upper traces) were recorded during the first minute of channel activity, the inward ones (2 lower traces) during the second minute.

ATP concentration of 2 mM the complete reverse blocking of channel develops in all the cases (Fig. 1A).

Current–voltage relations (Fig. 1B) demonstrated their saturation in the outward direction at the level of about 3 pA (in case of the physiological K^+ concentration relation at the outside ($[K^+]_o$) and inside ($[K^+]_i$) membrane surface, together with the inward rectification which is distinctly seen for the more symmetrical case in $[K^+]_i$. Here the single channel conductance value defined according over the linear part of I–V relation in average was equal to 99 ± 2 pS (mean ± SD, n = 4). In some experiments the sublevels with the less channel conductance (Fig. 1C) were registered, though under such conditions the possibility of their appearance was small.

The analysis of the current kinetic characteristics was complicated because of the presence as usual of several channels in one path. It was only in 4 experiments that we managed to record the activity of a channel. This example is shown in Fig. 2. It is seen that the peculiarity of the burst kinetics of the currents is a better expression of the transitions into short close states within the bursts for inward currents. The evaluation of kinetic parameters was complicated because of the extreme (τ ranging up to 1 min) run-down inactivation (Fig. 3). For the calculation of the channel open and closed state durations, distribution fragments of recordings of their activity only during the first minute were taken. In this case they were well approximated by one and a sum of two exponents for open and closed states, respectively. At the membrane potential of 0 mV, $[K^+]_o$ = 10 mM and $[K^+]_i$ = 140 mM τ_o value was equal (on average) to 65.6 ± 13.7 ms, τ_1 = 2.4 ± 0.5 ms and τ_2 = 39.0 ± 11.2 ms (mean ± SD, n = 4).

4. DISCUSSION

The comparison of the characteristics observed with the given ones for the analogous channels in the sarcolemma of cardiomyocytes of different animals show their likeness in the ATP concentration, sufficient for full channel blocking [1,5,13] and in differences of conductance and burst kinetics for inward and outward currents [1,2,14]. The analysed channels possess the $[K^+]_o$-dependent conductance which surpasses the value calculated according to the equation [7] corresponding

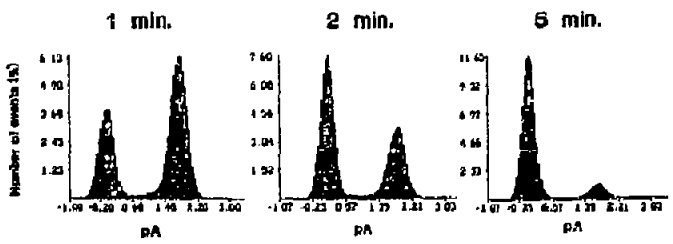

Fig. 3. The sequence of amplitude histograms illustrating the P_o decrease, conditioned by the run-down. Every histogram was obtained while analysing the fragments of uninterrupted patch current recording corresponding to the first, the second and the fifth minutes of its activity. The conditions of the registration of the outward currents, as for the case shown in Fig. 2, are holding current is equal to −8 mV

to the previously received data [2,15]. The presence of the run-down of these channels' activity (in comparison with other data [3,6,8]) may be estimated as the evidence of a still greater dependence of ATP-sensitive K^+ channels functioning upon the completeness of regulation mechanisms inherent to an intact cell. The registered conductance sublevels analogous to that previously found in the channels of this type in the guinea-pig ventricular cardiomyocytes sarcolemma [2] remind us again about the necessity of the carrying out the systematic analysis of this irregular property [7]. Besides it is impossible to overlook one more peculiarity of their functioning. On the one hand, the analysed channels have been found only in every fifth patch (on average). On the other hand, there were several channels in a greater portion of 'active' membrane patches (as has already been observed [8]). It would be natural to presume that it should be conditioned by the cluster localization of ATP-sensitive K^+ channels in the human adult heart cell membrane.

REFERENCES

[1] Noma, A , (1983) Nature 305, 147–148
[2] Kakei, M., Noma, A and Shibasaki, T. (1985) J Physiol. 363, 441–462.
[3] Findlay, I. (1987) Pfluegers Arch. 410, 313–320.
[4] Qin, D., Takano, M. and Noma, A. (1989) Am. J. Physiol. 257, H1624–H1633.
[5] Lederer, W.J. and Nichols, C.G. (1989) J. Physiol. 419, 193–211.
[6] Takano, M., Qin, D. and Noma, A. (1990) Am. J. Physiol. 258, H45–H50.
[7] Noma, A and Takano, M. (1991) Jap J. Physiol. 41, 177–187.
[8] Findlay, I. and Faivre, J.F. (1991) FEBS Lett. 279, 95–97.
[9] Babenko, A.P. and Samoilov, V.O. (1991) Fiziologicheskij zurnal SSSR 77, 55–59.
[10] Kakei, M. and Noma, A (1984) J. Physiol. 352, 265–284.
[11] Jacobson, S.L., Banfalvi, M. and Schwarzfeld, T.A. (1985) Basic Res. Cardiol. 80 (Suppl. 1), 79–82.
[12] Hamill, O P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) Pfluegers Arch. 391, 85–100.
[13] Findlay, I (1988) Pfluegers Arch 412, 37–41.
[14] Zilberter, Yu., Burnashev, N., Papin, A., Portnov, V. and Khodorov, B. (1988) Pfluegers Arch. 411, 584–589.
[15] Trube, G and Hescheler, J. (1983) Naunyn-Schmiedeberg's Arch. Pharmacol. 322, R64.