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ATP-sensitive K channels in heart muscle

Spare channels

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We show that ATP-sensitive K^+ channels of excised inside-out membrane patches of rat ventricular myocytes show considerable variation in their sensitivity to ATP. In 102 different membrane patches IC_{50} values ranged from 9 to 580 μM ATP and Hill coefficients from 1% to 6. 41% of patches showed openings of ATP-sensitive K^+ channels in the presence of 1 mM ATP. These results considerably widen the range of internal ATP concentrations over which one might expect activation of the ATP-sensitive K^+ current in cardiac myocytes.

K channel; Ligand sensitivity; ATP; Rat ventricular muscle

1. INTRODUCTION

The 'spare channel' hypothesis [1] was put forward for ATP-sensitive K^+ (K-ATP) channels in insulin-secreting B cells of mammalian islets of Langerhans to explain the discrepancy between the in vitro sensitivity of the channels to internal ATP and channel openings in presence of the supramaximal ATP content of intact B cells (see [2] for review). It suggests that K-ATP channels should show a range of sensitivity to internal ATP. Here we provide the first experimental support for this hypothesis by demonstrating considerable variation in the sensitivity of K-ATP channels recorded in excised membrane patches from rat ventricular myocytes.

2. MATERIALS AND METHODS

Individual myocytes were obtained from rat hearts with conventional methods [3]. Single ion channel records were obtained from excised inside-out membrane patches [4]. Each membrane patch was voltage-clamped at 0 mV with Na-rich solution (140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM Hepes, 10 mM glucose, pH 7.4) bathing the external and K-rich solution (140 mM KCl, 10 mM Hepes, 10 mM glucose, 5 mM EDTA, pH 7.4) bathing the internal face of the membrane. ATP (disodium salt) was obtained from Sigma (La Verpilliere, France). Control and test solutions were applied to patches from a series of parallel pipes. All experiments were conducted at room temperature (20–22°C). Ion channel open probability was estimated by comparing the actual patch K-ATP current with the maximum possible patch K-ATP current [5]. Experiments were confined to membrane patches which showed high open probability channel activity in control conditions and which did not show 'run-down' in the time scale of these experiments [6]. Estimates of IC_{50} values, Hill coefficients and lines on graphs were obtained by fitting individual data with an unweighted non-linear least squares gradient-expansion algorithm of Marquardt to the following equation:

$$1/1+(10^{[ATP]\cdot H}/IC_{50}^{(H)})$$

where ATP represents concentration, H the Hill Coefficient and IC_{50} the concentration of ATP evoking 50% channel closure.

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3. RESULTS

In this study of 102 different membrane patches the average number of K-ATP channels per patch was 5.6 ± 0.6 (± SE) and the average channel open probability recorded in the absence of ATP was 0.82 ± 0.01 (± SE). Since only five of these membrane patches contained one K-ATP channel our analysis has been largely based upon multiple channel patches. In these examples the calculation of the Hill coefficient will be an underestimate if ion channels of different sensitivity to the ligand are present in the same patch. Fig. 1 provides an example of the differences in sensitivity to ATP that we recorded from different membrane patches. Although these patches showed similar high open probability activity of K-ATP channels in the absence of ATP, 0.921 (Fig. 1A) and 0.877 (Fig. 1B), their responses to ATP were markedly different and completely reversible (not shown). The patch shown in Fig. 1A had an IC_{50} value of 16 μM ATP and a Hill coefficient of 2.7, the patch in Fig. 1B had an IC_{50} of 70 μM ATP and a Hill coefficient of 2.2 (Fig. 1C).

Fig. 2 illustrates the effect of ATP upon channel open probability in the 102 membrane patches obtained from 27 rats. The line represents an IC_{50} value of 37 μM ATP and a Hill coefficient of 1.94. In individual mem-

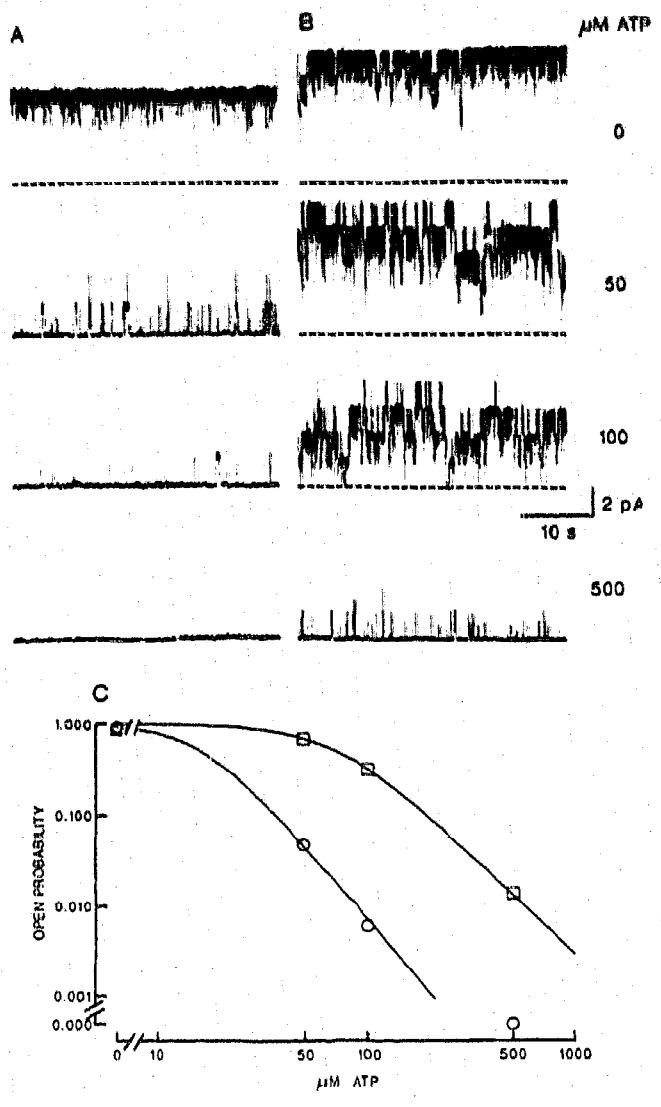

Fig. 1. Dose-dependent inhibition of K-ATP channels by internal ATP. (A) This patch contained 3 K-ATP channels. (B) This patch contained 5 K-ATP channels. These patches had been isolated from the same heart and were exposed to identical control and test solutions. The concentration of ATP applied to the patches is shown to the right of the figure. The dotted lines indicate the membrane patch current level when all K-ATP channels were closed. (C) Log-log representation of the effect of ATP upon K-ATP channel open-probability. The circles represent the patch shown in (A); the squares represent the patch shown in (B).

brane patches the IC_{50} values ranged from 9 to 580 μM ATP and Hill coefficients from 1.4 to 5.6. It should be noted that 21 of 51 membrane patches showed channel openings in the presence of 1 mM ATP. Of the five membrane patches which contained a single K-ATP channel the estimated IC_{50} values and Hill coefficients were 20, 29, 60, 69, 161 μM ATP and 2.5, 1.3, 3.9, 3.0, 5.6, respectively.

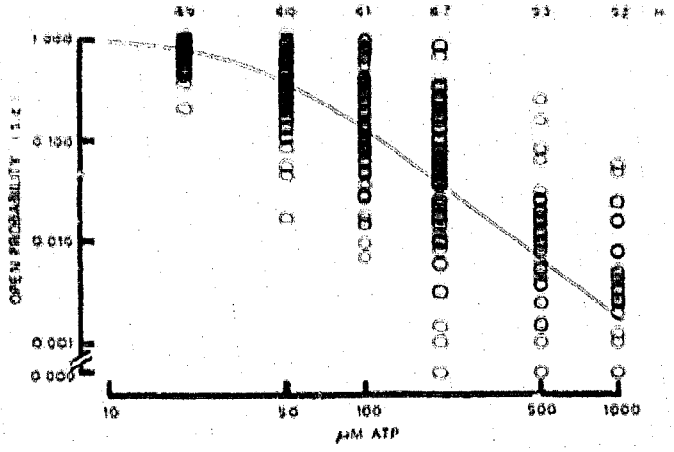

Fig. 2. Dose/response relationship between openings of K-ATP channels and internal ATP concentration. The circles represent individual recordings of K-ATP channels where channel open probability in the presence of ATP is expressed relative to that recorded in the absence of ATP (i/c). This graph contains the results obtained from 102 different membrane patches. The values above each column of individual results (n) represent the number of different membrane patches exposed to the corresponding concentration of ATP. Each membrane patch was exposed to 3 or 4 different concentrations of ATP.

4. DISCUSSION

The IC_{50} value of 37 μM ATP estimated from all the data obtained here is within the range of 17–100 μM ATP reported for the dose/response relationship between internal ATP and closure of the cardiac muscle K-ATP channel [7–11]. Until now little attention has been paid to the variability of individual results although this has been noted [12]. We show here that different patches can exhibit up to a 60-fold difference in the concentration of ATP required to inhibit channel opening by 50%. These experimental data therefore provide the first direct support for the 'spare channel' hypothesis of Cook et al. [1] and apply it to cardiac muscle. Fundamental to the 'spare channel' hypothesis in the B-cell is a low membrane conductance which allows the modulation of the low open probability of the K-ATP channel to regulate the cell membrane potential [1,2]. This is not the case for cardiac ventricular muscle where the myocytes maintain a relatively high resting permeability to K^+; on the other hand, the plateau of the ventricular action potential is characterised by a low membrane conductance [13]. This explains why activation of the K-ATP current reduces action potential duration without significantly affecting other ventricular electrical parameters [14,15].

The interpretation of the Hill coefficient as indicating the number of ligand molecules associated with the receptor has recently been questioned [12] where the kinetics of ATP-induced closure suggested a Hill coefficient of 1 whereas steady-state channel inhibition was associated with a Hill coefficient of 2. In this study the population estimate obtained under steady-state condi-

tions was 2 though in individual membrane patches the coefficient ranged from close to 1 to nearly 6.

Both experimental [15,16] and theoretical [9] evidence points to a very small proportion of the myocytes' K-ATP current (approximately 1%) being responsible for significant shortening of action potential duration. With 'spare channels' this would result from the opening of a very few of the myocytes' K-ATP channels. We show that this can occur in the presence of mM intracellular ATP (Fig. 2). Further activation of the K-ATP current would involve not only an increase in individual channel open probability but also the progressive recruitment of channels. This provides a control mechanism over a wide range of concentrations of internal ATP. At the other extreme even during myocyte contracture induced by metabolic inhibition not all K-ATP channels open [16]. It is thus clear that the wide range of sensitivity to ATP observed here finds correlates under physiological and pathological conditions.

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REFERENCES

[1] Cook, D.L., Satin, L.S., Ashford, M.L.J. and Hales, C.N. (1988) Diabetes 37, 495–498.
[2] Ashcroft, S.J.H. and Ashcroft, F.M. (1990) Cellular Signalling 2, 197–214.
[3] Mitra, R. and Morad, M. (1985) Am. J. Physiol. 249, H1056–H1060.
[4] Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) Pfluegers Arch. 391, 85–100.
[5] Findlay, I. (1988) Biochim. Biophys. Acta 943, 297–304.
[6] Findlay, I. (1987) Pfluegers Arch. 410, 313–320.
[7] Findlay, I. (1988) Pfluegers Arch. 412, 37–41.
[8] Lederer, W.J. and Nichols, C.G. (1989) J. Physiol. (Lond.) 419, 193–211.
[9] Nichols, C.G. and Lederer, W.J. (1990) J. Physiol. (Lond.) 423, 91–110.
[10] Noma, A. (1983) Nature (Lond.) 305, 147–148.
[11] Thuringer, D. and Escande, D. (1989) Mol. Pharmacol. 36, 879–902.
[12] Qin, D., Takano, M. and Noma, A. (1989) Am. J. Physiol. 257, H1624–H1633.
[13] Weidmann, S. (1951) J. Physiol. (Lond.) 115, 227–236.
[14] Hiraoka, M. and Fan, Z. (1989) J. Pharmacol. Exp. Ther. 250, 278–285.
[15] Findlay, I., Deroubaix, E., Guiraudou, P. and Coraboeuf, E. (1989) Am. J. Physiol. 257, H1551–H1559.
[16] Faivre, J.-F. and Findlay, I. (1990) Biochim. Biophys. Acta 1029, 167–172.