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Cytosolic ADP enhances the sensitivity to tolbutamide of ATP-dependent K^+ channels from pancreatic B-cells

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The effects of intracellular purine nucleotides on tolbutamide-induced block of ATP-dependent K^+ channels from mouse pancreatic B-cells were studied using the patch-clamp technique. When applied to the inside of excised patches, tolbutamide alone blocked channel activity half-maximally at 55 μM and the concentration-response curve for the inhibition of K^+ channels by tolbutamide was flat. ADP (1 mM), but not other nucleotides (AMP, GTP or GDP) increased the steepness of the concentration-response curve and decreased the half-maximally effective tolbutamide concentration to 4.2 μM. It is suggested that the ATP-dependent K^+ channel or a closely related structure contains a receptor which is accessible for cytosolic ADP and controls the sensitivity to tolbutamide.

K^+ channel; Tolbutamide; Purine nucleotide; (Pancreatic B-cell)

1. INTRODUCTION

Glucose and sulfonylureas are believed to initiate electrical activity and, subsequently, insulin release by inhibiting the same type of K^+ channel in the B-cell membrane [1]. Glucose regulates the channel indirectly, probably via changing the intracellular ATP/ADP ratio. ATP is a direct channel blocker, whereas ADP or GDP were found to relieve ATP-induced inhibition [2–6]. A couple of recent publications suggested that hypoglycemic sulfonylureas block the K^+ channel both in the presence and in the absence of ATP [7–11]. Therefore, we investigated whether intracellular nucleotides alter the sensitivity to sulfonylureas of the ATP-dependent K^+ channel.

2. MATERIALS AND METHODS

Pancreatic B-cells were isolated from NMRI mice and kept in culture for 1–2 days [12]. The whole-cell and the inside-out configuration of the patch-clamp technique [13] were employed to record currents flowing through ATP-dependent K^+ channels. An EPC7-patch-clamp amplifier was used for voltage control and current amplification. Signals were displayed on an *oscilloscope or on a chart recorder (220, Gould, Cleveland,* OH, USA). Traces shown in the publication were photographed from the paper recordings. In both patch-clamp configurations the solution (A) at the cytoplasmic side of the membrane contained (concentrations in mM): 140 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 5 Hepes and 10 EGTA (pH 7.15). For the whole-cell experiments, *the extracellular solution (B) was composed of (mM): 5.6 KCl,* 140 NaCl, 2.6 $CaCl_2$, 1.2 $MgCl_2$ and 10 Hepes (pH 7.40). NaCl was equimolarly replaced by KCl in the solution (C) at the extracellular side of inside-out patches. Na_2ATP, KADP, Na_2AMP, Na_2GTP or Li_2GDP were added to solution A to give the final concentrations stated in the text or legends and pH was readjusted to 7.15 if necessary. Free (Mg^{2+}) was held constant at 0.7 mM by adding appropriate amounts of $MgCl_2$ to the nucleotide-containing solutions. The pipette potential in inside-out experiments was held at +50 mV. ATP (1 mM) was normally present at the cytoplasmic face of excised patches to reduce the run-down of channel activity and ATP-free solutions containing the test substances were applied for 10 s intervals at a rate of 0.033 Hz [14]. The holding potential in whole-cell experiments was −70 mV and small voltage pulses (10 mV, 200 ms, rate 0.5 Hz) were given repetitively to measure the *input resistance and capacitance of the cell (10). Experiments* were carried out at room temperature (20–22°C).

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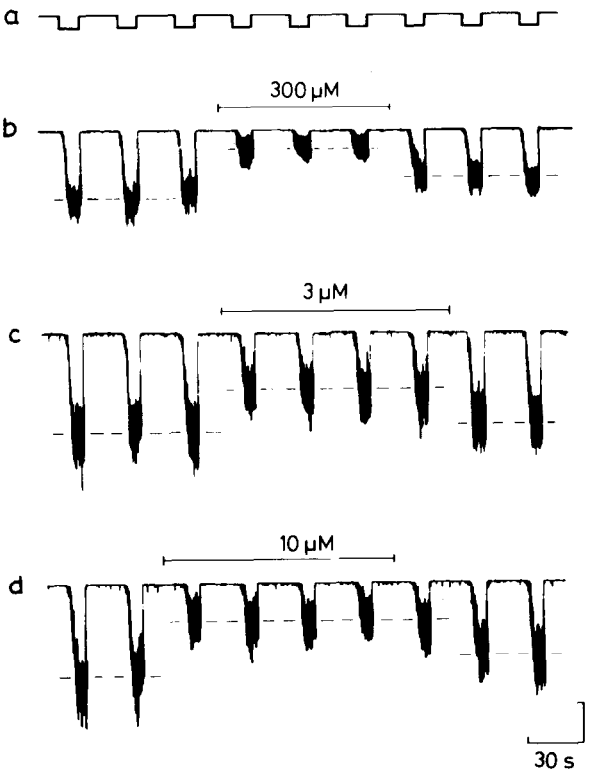

Fig.1. Tolbutamide-induced block of ATP-dependent K^+ channels in an inside-out patch. (a) Schematic protocol of solution exchange. ATP (1 mM) was present at the high level and absent at the low level of this trace. (b–d) Current recordings. Since channel activity declined with time (run-down), the amplifier gain was increased between traces b and c (vertical calibration bar: 40 pA in b, 20 pA in c and d). Horizontal bars in b–d indicate application of tolbutamide (300 μM, b; 3 μM, c; 10 μM, d). Tolbutamide was only added to the solution without ATP. Dashed lines in b–d represent estimates of mean current amplitudes during the pulses of test solution (0 mM ATP).

3. RESULTS

Fig.1 shows an example of tolbutamide-induced block of ATP-dependent K^+ currents recorded from an inside-out patch. The inhibitory effect of 3 and 10 μM tolbutamide (traces c and d) was approximately as expected from previous whole-cell experiments [8,10]. A concentration of 300 μM, however, suppressed channel activity by only 56% (trace b), whereas 98% block had been found in the whole-cell experiments [8,10]. Fortuitously we discovered that ADP (1 mM) strongly improved the blocking intensity of tolbutamide (fig.2, cf. traces b and d). At a 10-fold lower concentration ADP had little effect on the tolbutamide-induced block of the ATP-dependent K^+ current (cf. traces c and d). Application of ADP (1 mM) alone resulted in moderate channel inhibition in the experiment of fig.2 (cf. traces b and d, pulses in the absence of tolbutamide), but a slight enhancement of channel activity was also seen in other experiments (still, block by tolbutamide was improved). This variability of ADP effects has already been described [3]. Concentration-response curves for the inhibition of the ATP-sensitive K^+ current by tolbutamide are shown in fig.3. The relation obtained from the inside-out patches in the absence of ADP was flat (Hill coefficient $n = 0.29$) and 50% effect were reached at a high concentration ($EC_{50} = 55$ μM). Presence of 1 mM ADP increased the steepness of the concentration-response curve ($n = 1.1$) and shifted the EC_{50} to 4.2 μM. The effects of ADP on tolbutamide-induced channel block seemed to be specific for this nucleotide, since AMP, GTP or GDP did not improve the sensitivity of ATP-dependent K^+ channels to tolbutamide (not shown). In the absence of nucleotides tolbutamide (100 μM) blocked channel activity by 51% (fig.3). Similarly weak inhibition was reached at this concentration in the presence of AMP (50%, $n = 3$ experiments), GTP (39%, $n = 2$) or GDP (50%, $n = 4$; channel activity in the absence of sulfonylurea, but the presence of the tested nucleotide was always defined as 100%). AMP or GTP alone did not markedly affect openings of K^+ channels, whereas GDP nearly doubled channel activity. Due to the strong intrinsic blocking effect of ATP a possible modulatory influence of this nucleotide on the tolbutamide sensitivity could only be examined at a low ATP concentration (20 μM). Under this condition, block by tolbutamide (100 μM) was still weak (40%, $n = 4$).

The situation in the whole-cell configuration resembled that in inside-out experiments. In the absence of nucleotides in the pipette solution tolbutamide (100 μM), when applied either to the intracellular or to the extracellular side of the membrane, reduced the cells resting conductance only by 40–50% (table 1). The same tolbutamide concentration strongly blocked the input conduc-

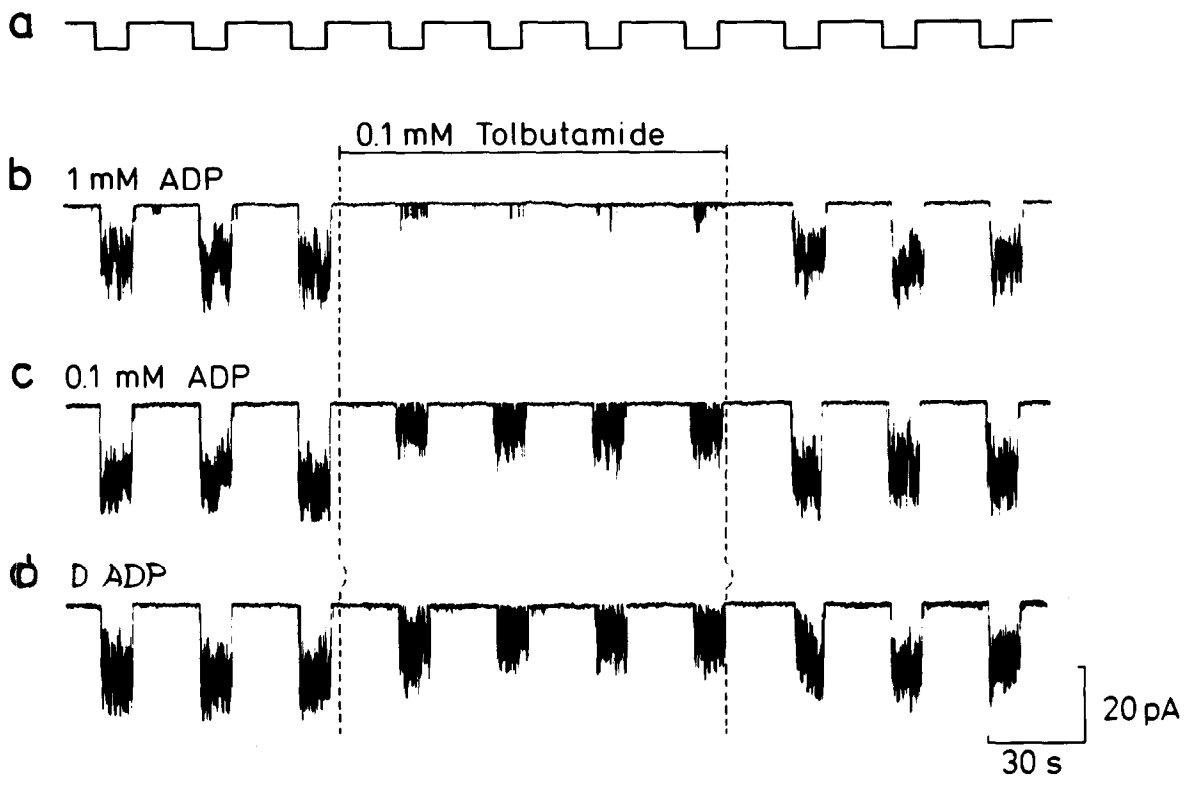

Fig.2. Effects of ADP on tolbutamide-induced block of ATP-dependent K^+ channels. (a) Schematic protocol of solution exchange as in fig.1. (b–d) Current traces obtained from an inside-out patch. ADP (1 mM, b; 0.1 mM, c; 0 mM, d) was applicated during pulses of ATP-free solutions. Horizontal bar above b–d indicates applications of tolbutamide (0.1 mM) together with ADP. Traces are shown in the sequence of recording during the experiment.

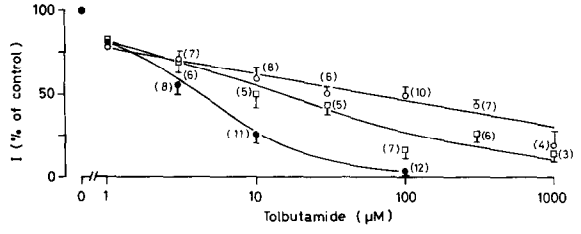

Fig.3. Effect of ADP on the relation between ATP-sensitive K^+ current and tolbutamide concentration in inside-out patches. Tolbutamide and ADP were applied to the intracellular side of the membrane. Time-averaged current I is plotted as a percentage of the current in the absence of tolbutamide, but in the presence of ADP. Symbols (○, 0 mM ADP; □, 0.1 mM ADP; ●, 1 mM ADP) and bars indicate mean values and SE, respectively. Numbers of observations are given in parentheses (bars and numbers of observations are not shown for 1 μM tolbutamide). Curves fitted to the measured values by a non-linear least-squares routine follow the function

$$I = 100 - \frac{100 \cdot [A]^n}{[EC_{50}]^n + [A]^n}$$

with $[A]$ = tolbutamide concentration, $[EC_{50}]$ = half-maximally effective concentration and n = slope parameter (Hill coefficient). The following values were obtained for $[EC_{50}]$ and n: 55 μM and 0.29 in the absence of ADP; 13.8 μM and 0.51 in the presence of 0.1 mM ADP; and 4.2 μM and 1.13 in the presence of 1 mM ADP.

tance if 1 mM ADP was present in the pipette solution. It should be noted that previous whole-cell experiments showing a similarly high activity of tolbutamide were performed with 0.3 or 1 mM ATP in the intracellular solution [8,10].

Table 1

Effects of ADP on tolbutamide-induced K^+-current block in whole-cell experiments

Intracellular sol. (pipette)	Extracellular sol. (bath)	pA/pF
–	–	27.0 ± 1.7 (76)
100 μM tolb.	–	16.5 ± 2.3[a] (20)
–	100 μM tolb.	13.8 ± 2.1[a] (19)
1 mM ADP	–	14.2 ± 2.1 (18)
1 mM ADP + 100 μM tolb.	–	0.2 ± 0.1 (9)
1 mM ADP	100 μM tolb.	0.1 ± 0.1 (8)

[a] Values for tolbutamide (100 μM) applied either to the extracellular or to the intracellular side of the membrane did not differ significantly ($p > 0.05$, two-tailed U-test of Wilcoxon and of Mann and Whitney)

Pipette solution (Section 2; A) and bath solution (B) contained tolbutamide or ADP as indicated. Current amplitudes evoked by 10 mV voltage pulses were divied by the cell capacitance, which is proportional to the cell surface area. Results represent mean values ± SE with numbers of experiments given in parentheses

4. DISCUSSION

The present study shows that intracellular ADP increases the sensitivity of ATP-dependent K^+ channels to tolbutamide. The presence of ADP but not of other nucleotides seems necessary to obtain a steep concentration-response curve for the sulfonylurea (Hill coefficient ≈1). Recently, it has been shown that ADP reduces the block of the channel by ATP and it was suggested that both nucleotides compete for the same binding site [4,5]. It is unclear whether the interference between ADP and tolbutamide is also due to binding of ADP to the ATP site. The concentration-response curve for tolbutamide obtained from the inside-out patches in the presence of ADP (1 mM, fig.3, filled circles) is very similar to the concentration-response curves from whole-cell experiments with ATP (0.3–1 mM) in the pipette solution [8,10], whereas the effect of tolbutamide (100 μM) was weak when both nucleotides were missing in the pipette (table 1). This does not necessarily mean, however, that ATP itself supports inhibition by tolbutamide. Alternatively, part of the ATP in the pipette solution may have been hydrolyzed to ADP by enzymes remaining in the cell during the whole-cell experiments. This hypothesis could also explain why ATP is more potent in blocking channels in inside-out patches than in inhibiting the resting K^+ conductance in whole-cell experiments [8,10].

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