The ATP-sensitivity of K^+ channels in rat pancreatic B-cells is modulated by ADP

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ATP-sensitive K^+ channels in inside-out membrane patches from dispersed rat pancreatic B-cells were studied using patch-clamp methods. The dose-response curve for ATP-induced channel inhibition was shifted to higher concentrations in the presence of ADP (2 mM). In glucose-free solution, the total intracellular concentration of ATP was 3.8 mM and of ADP 1.5 mM; glucose (20 mM) increased ATP and decreased ADP by approx. 40%. These results suggest that both ADP and ATP may be involved in regulating the activity of the glucose-sensitive K^+ channel in intact B-cells.

(Pancreatic B-cell) *K^+ channel* *Insulin secretion*

1. INTRODUCTION

A central event in glucose-stimulated insulin release from the pancreatic B-cell is depolarisation of the B-cell membrane; the resulting increase in Ca^{2+} influx through voltage-dependent Ca^{2+} channels leads to insulin secretion [1]. Although it has recently become clear that the initial depolarisation results from the closing of specific K^+ channels as a consequence of glucose metabolism [2,3], the link between B-cell metabolism and channel inhibition remains unresolved. Two pieces of evidence support a role for ATP. First, in inside-out patches the glucose-sensitive K^+ channel is inhibited by ATP [3–6]. Secondly, changes in $[ATP]_i$ produced by glucose and by metabolic inhibitors are accompanied by reciprocal changes in the channel open probability [6,7]. However, there remains an apparent discrepancy between the ATP sensitivity of the channel in the intact cell and that found for the excised patch. In intact cells, the ATP-sensitive channel is open in glucose-free solutions [2,3] in which $[ATP]_i$ is around 5 mM [6–9], whereas a K_i of 15 μM is measured for isolated membrane patches [4]. We now report that ADP modulates the ATP sensitivity of the channel.

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2. MATERIALS AND METHODS

Single pancreatic B-cells were isolated from normal adult rat islets of Langerhans and maintained in short-term tissue culture (1–5 days) [2,7].

Standard patch-clamp methods [10] were used to record single-channel currents from inside-out membrane patches. The patch pipette contained (mM): 140 KCl, 2 $CaCl_2$, 5 Na-Hepes (pH 7.4). The intracellular (bath) solution contained (mM): 107 KCl, 1 $CaCl_2$, 5 $MgSO_4$, 11 EGTA + 11 Hepes (stock solution titrated to neutrality with KOH), pH 7.2; total $[K]_i$ was about 135 mM. K_2ATP, KADP and NaAMP were added as indicated. Intracellular solutions were rapidly changed by moving the pipette tip into the solution flowing from one of the mouths of a series of tubes [11]. All experiments were carried out at room temperature (20–25°C). Currents were recorded using a List EPC5 patch-clamp amplifier onto FM tape (bandwidth 10 kHz at −1 dB). For analysis of the extent of channel activity (*NP*) current records were subsequently low pass filtered at 1 kHz (8-pole

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Bessel, −3 dB value) amplified × 10 and digitised at 5 kHz using a 12-bit ADC controlled by a PDP 11/73 computer. Channel activity (*NP*) was calculated from $I = NPi$, where I is the mean value of the current during the recording period, i is the amplitude of the single-channel current, N is the number of active channels and P is the channel open probability. The average current was calculated by integrating the total current (after subtraction of the baseline) and dividing by the total time of the sample (typically 20–30 s of data at each concentration).

For measurement of adenine nucleotides, batches of 8000 dispersed B-cells were incubated in Eppendorf tubes in 0.5 ml Hepes-buffered bicarbonate medium [12] containing albumin (2 mg/ml) and in the presence and absence of 20 mM glucose. After 45 min at room temperature, tubes were centrifuged for 5 min at 300 × *g*. All but 10 µl of the supernatant was aspirated and cells extracted by the addition of 50 µl ice-cold 2.5% perchloric acid and mechanical vibration. Extracts were neutralised with 30 µl ice-cold 1 M $KHCO_3$ and 20 µl of 400 mM Hepes buffer (pH 7.4) containing 25 mM $MgCl_2$, 0.5 M KCl and 5 mM phospho*enol*-pyruvate was added. The precipitates were sedimented by centrifugation for 1 min in a Beckman microfuge and 100 µl of the supernatant transferred to a new tube. Two aliquots of 25 µl each were taken for assay of ATP. To the remainder was added 0.5 U pyruvate kinase in 5 µl of the same buffer to convert ADP to ATP. After 30 min at room temperature a further two 25 µl aliquots of the reaction media were taken for assay of (ADP + ATP) as ATP. ATP was measured by a firefly luciferase method [8]. Concentrations of

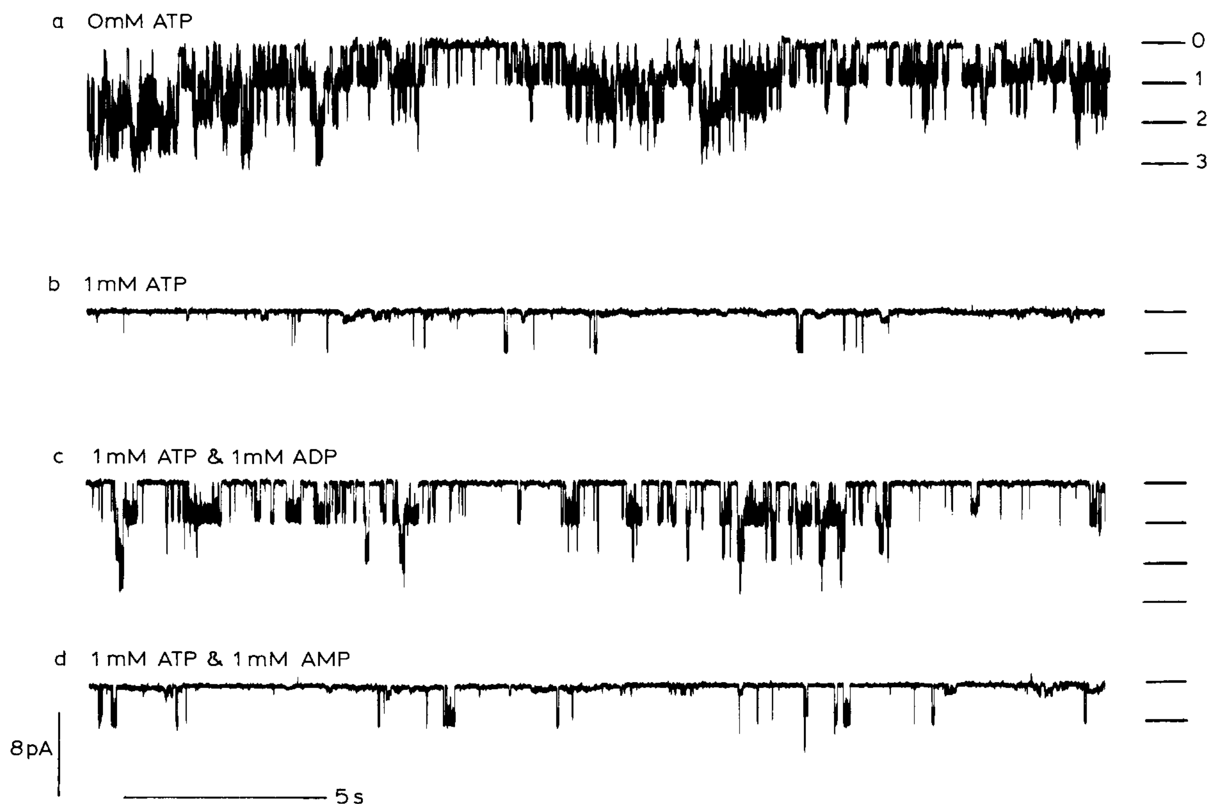

Fig.1. Effect of adenine nucleotides on single K^+ channel currents recorded at −60 mV from an inside-out patch exposed to control solution (0 mM ATP, a) and about 2 s after exposure to an intracellular solution containing 1 mM ATP (b), 1 mM ATP + 1 mM ADP (c) or 1 mM ATP + 1 mM AMP (d). The current level when 0, 1, 2, or 3 channels are open is indicated to the right of the trace. Records are displayed using a Gould 2400 chart recorder (filter, 0.3 kHz). In this experiment the values for *NP* were: a, 0.766; b, 0.048; c, 0.285; d, 0.049.

ADP and ATP were estimated using a B-cell volume of 1.15 pl calculated from the measured mean B-cell diameter of 13 μm [7].

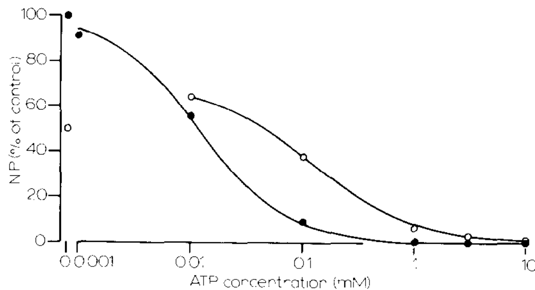

Fig.2. Effect of 2 mM ADP on the dose-response curve for the inhibitory action of ATP on the average current measured for a single patch. (●) 0 mM ADP, (○) 2 mM ADP. Channel activity (*NP*) is plotted as a percentage of that in the control solution (i.e. no ATP or ADP). Solutions containing ATP and/or ADP concentrations greater than 1 mM were adjusted to maintain a constant $[K]_i$. Membrane potential, -60 mV. The curves are drawn to the equation

$$y = y_{max} - y_{max}/(1 + x/K_i)^H$$

where K_i is the ATP concentration giving half-maximal inhibition and H is the Hill coefficient. In the absence of ADP, $y_{max} = 100$, $K_i = 12\ \mu$M and $H = 1.1$ (correlation coefficient of 0.996 when fitted using linear regression analysis to the logarithm of the data). In the presence of 2 mM ADP, $y_{max} = 64$, $K_i = 145\ \mu$M and $H = 1$ (correlation coefficient, 0.993). In many patches it was not possible to construct an ATP dose-response curve either because of the simultaneous presence of a 20 pS channel or because of a decrease in the activity of the ATP-sensitive channel with time (rundown). The data shown are typical of 3 similar experiments for patches in which the 20 pS channel was absent and in which the activity of the ATP-sensitive channel did not decline with time.

3. RESULTS

Fig.1a shows single K^+ channel currents recorded at -60 mV in the absence of adenine nucleotides. The addition of 1 mM ATP to the cytoplasmic membrane surface (b) reduced the degree of channel activity (*NP*) by about 95% (from 0.77 to 0.048). Channel activity was partially restored (*NP* = 0.29) by the subsequent addition of 1 mM ADP to this solution (c). This result was observed in each of 7 patches. However, AMP did not reverse channel inhibition by ATP at either 1 mM (d, *NP* = 0.049; and 2 other patches) or 5 mM (2 patches). Adenosine (5 mM, $n = 2$) and phosphate (5 mM, $n = 3$) were also ineffective.

The dose-response curves for inhibition by ATP in the absence and presence of 2 mM ADP are shown in fig.2. In this patch, the half-maximal inhibitory concentration of ATP was 12 μM; the line drawn through the data points has a Hill coefficient of 1.1. Fig.2 also shows that ADP, in the absence of ATP, can inhibit channel activity but is considerably less effective than ATP (50% inhibi-

Table 1

Effect of glucose on the concentration of adenine nucleotides

	[ATP] (mM)	[ADP] (mM)	[ATP]/ [ADP]
0 mM glucose	3.8 ± 0.2	1.5 ± 0.1	2.85 ± 0.12
20 mM glucose	5.3 ± 0.2[a]	1.0 ± 0.1[a]	5.52 ± 0.4[a]
% change	+39	−39	+94

[a] $p < 0.001$

Total B-cell concentrations of ATP and ADP were measured by a luciferase method as described in section 2. Results are expressed as mean ± SE for 7 observations

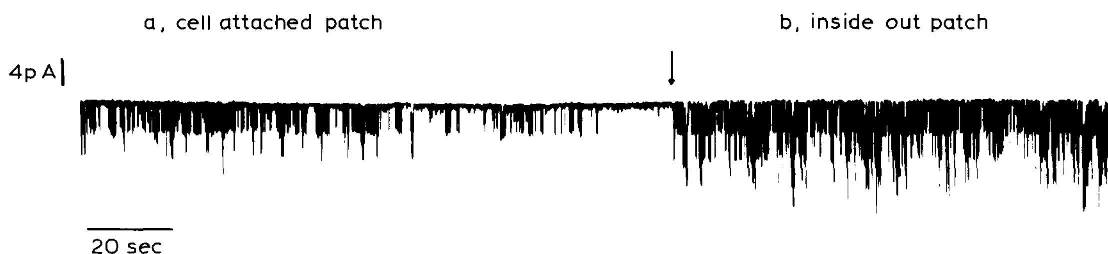

Fig.3. Single-channel currents recorded at a membrane potential of -60 mV before and after patch excision. The bath contained an intracellular solution plus 4 mM ATP and 1.5 mM ADP.

tion by 2 mM ADP as compared with 12 μM ATP). In the presence of 2 mM ADP, the ATP dose-response curve was shifted to higher concentrations with half-maximal inhibition at 145 μM ATP; there was no change in the Hill coefficient. These results are representative of 3 similar experiments.

Fig.3 shows that channel activity recorded from a cell-attached patch on an intact B-cell incubated for 1 h in the absence of glucose did not decrease when the patch was excised into a solution containing 4 mM ATP and 1.5 mM ADP. These nucleotide concentrations were chosen to approximate their measured total intracellular concentrations under the same experimental conditions, as shown in table 1. This table also shows that 20 mM glucose produced an increase in ATP and a decrease in ADP.

4. DISCUSSION

Inhibition of the ATP-sensitive K^+ channel may underlie the glucose-induced depolarisation of the B-cell membrane that leads to Ca^{2+} influx and the initiation of insulin release [2,3]. Consistent with previous studies [3–6], our results confirm that in inside-out patches this K^+ channel is inhibited by micromolar concentrations of ATP. We also show that in our dispersed B-cell preparation the total intracellular concentrations of ATP and ADP lie in the millimolar range, as reported for islets of Langerhans [8,9]. The major new observation is that ADP at these concentrations (1–2 mM) can relieve ATP-induced channel inhibition. This effect may explain, at least partly, why channel activity can be recorded from intact cells. We are aware that because of possible compartmentation of ATP and/or ADP the concentrations of these adenine nucleotides at membrane sites may be different from those we measure. Nevertheless, channel activity was not decreased by excision into a solution containing ATP and ADP at concentrations similar to those measured in resting B-cells.

Since we find that glucose both increases ATP and decreases ADP in dispersed B-cells (as found for intact islets [9]), our results are also consistent with the idea that changes in the intracellular concentrations of both these nucleotides may contribute to the inhibition of the channel produced by glucose metabolism.

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