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Intracellular ADP activates K^+ channels that are inhibited by ATP in an insulin-secreting cell line

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The effect of ADP on ATP-sensitive K^+ channels in the insulin-secreting RINm5F cell line has been investigated with the help of single-channel current recording from saponin-permeabilized cells. ADP (100–500 μM) markedly activates K^+ channels when added to the bath solution in contact with the membrane inside. ADP-β-S cannot mimick this effect. During sustained ATP (500 μM)-evoked inhibition of K^+ channel opening, 500 μM ADP markedly and reversibly activates the channels. Conversely ATP markedly reduces the opening probability of ADP-activated channels. It is suggested that the physiological control of K^+ channel opening in the insulin-secreting cells is mediated by changes in ATP/ADP ratio rather than being solely determined by the ATP concentration.

(RINm5F cell) *Patch-clamp* *K^+ channel* *ADP* *ATP*

1. INTRODUCTION

K^+ channels that can be inhibited by intracellular ATP were discovered in cardiac cells [1] and later also found in pancreatic islet cells [2]. In the insulin-secreting cells these channels are particularly important as they are responsible for the resting potential [3–5] and they play a crucial role in stimulus-secretion coupling since it is the glucose or glyceraldehyde-evoked closure of these channels [4–6] that causes the membrane depolarization [7] which is required for opening the voltage-gated Ca^{2+} channels responsible for the characteristic action potentials [7–9]. Although it is now clear that the ATP-sensitive K^+ channels in the unstimulated islet cells have a low open-state probability due to the action of intracellular ATP [3], it may still seem surprising that any channels are ever open since in excised inside-out membrane patches 1 mM ATP abolishes all channel activity [2] whereas the resting intracellular ATP concentration is about 3–4 mM [10]. We now report that ADP in the concentration range 100–500 μM evokes immediate and fully reversible activation of partially run-down K^+ channels and most importantly markedly stimulates channel opening during ATP-evoked inhibition. This excitatory effect of ADP therefore has to be added to the inhibitory one already described for ATP [2] in order to understand the activity pattern in intact cells and the glucose-evoked increase in the ATP/ADP ratio [11] may explain the marked inhibition of K^+ channel opening previously demonstrated in intact cells [4–6].

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Abbreviations: ATP, adenosine 5′-triphosphate; ADP, adenosine 5′-diphosphate; ADP-β-S, adenosine 5′-*O*-(2-thiodiphosphate); AMP, adenosine 5′-monophosphate; cyclic AMP, adenosine 3′:5′-cyclic monophosphate

2. MATERIALS AND METHODS

The insulin-secreting cell line RINm5F [12] was used for all the experiments. We recorded single-channel currents [13] in the open cell-attached configuration [14–16] where the plasma membrane, apart from the isolated patch membrane covered

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by the recording micropipette, is permeabilized by a brief exposure of the cell to saponin (fig.1). This preparation is similar to the excised inside-out patch configuration in that it allows easy access to the intracellular aspect of an isolated membrane area from which single-channel currents are recorded, but has the advantage that the run-down of ATP-sensitive K^+ channels, observed in excised inside-out patches from several cell types [3,15,17], is occurring much more slowly [15].

3. RESULTS

Fig.1. shows an experimental record starting out with the recording pipette attached to an intact cell. In this situation there is normally only modest channel activity, but a brief exposure to saponin evokes a large increase in outward current which can be immediately and almost completely inhibited by bath application of 500 μM ATP. This effect is quickly reversible. When the open cell is thereafter exposed to 500 μM ADP there is a marked and immediate increase in the outward current which is again fully reversible upon wash-out of ADP. Such ADP-evoked channel activation was repeatedly observed in 11 separate permeabilized cells (n = 26). Smaller activations were observed with 100 μM ADP (n = 3) and with 250 μM ADP (n = 4). It has previously been reported that ADP has a weak inhibitory effect on channel opening [1,2] although this was not observed in other experiments [18]. In 4 of the above mentioned 11 cells ADP did in fact evoke inhibition in the first part of the experiment when the channel activity was high (fig.2A), but later after channel run-down had occurred clear activating effects were seen (fig.2C). In between, ADP evoked a biphasic effect consisting of first inhibition and then activation (fig.2B). When ADP was removed rapid decrease in activity followed (figs 1,2). In 3 other permeabilized cells ADP only evoked inhibition similar to that shown in fig.2A and in one further cell investigated in the same way no effects (inhibitory or excitatory) could be observed.

When the non-hydrolysable ADP analogue ADP-β-S (250–500 μM) was used instead of ADP

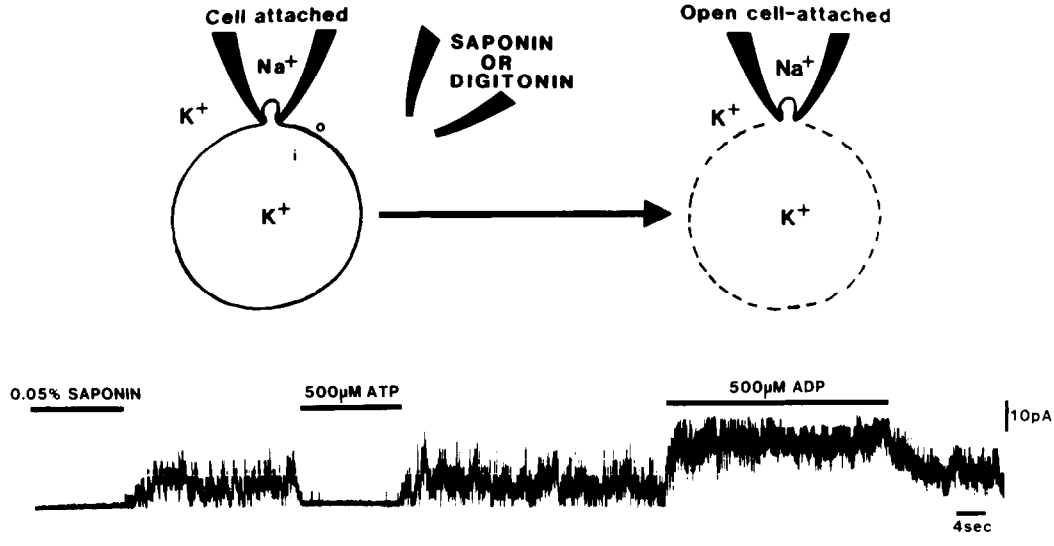

Fig.1. A continuous cell-attached membrane patch current record obtained from an individual RINm5F cell. The scheme illustrates the recording configurations. The patch pipette was filled with an Na^+-rich extracellular type of physiological saline solution containing (mM): 140 NaCl, 4.7 KCl, 1.13 $MgCl_2$, 2.5 glucose, 10 Hepes and 1.0 EGTA, no Ca^{2+} was added and the pH set at 7.2 using NaOH. The bath fluid was a K^+-rich intracellular type of physiological saline solution containing (mM): 140 KCl, 10 NaCl, 1.13 $MgCl_2$, 2.5 glucose, 10 Hepes and 0.5 EGTA, no Ca^{2+} was added and the pH set at 7.2 using KOH. During the period indicated the cell was permeabilized by gently blowing a 0.05% (w/v) solution of saponin into the stream of K^+-rich solution bathing the cell. During the periods indicated by the bars labelled ATP and ADP, the K^+-rich solution bathing the cells was switched to a K^+-rich solution containing 500 μM ATP and 500 μM ADP, respectively. The pipette voltage was clamped throughout at 0 mV and the current trace was filtered at 500 Hz (low pass).

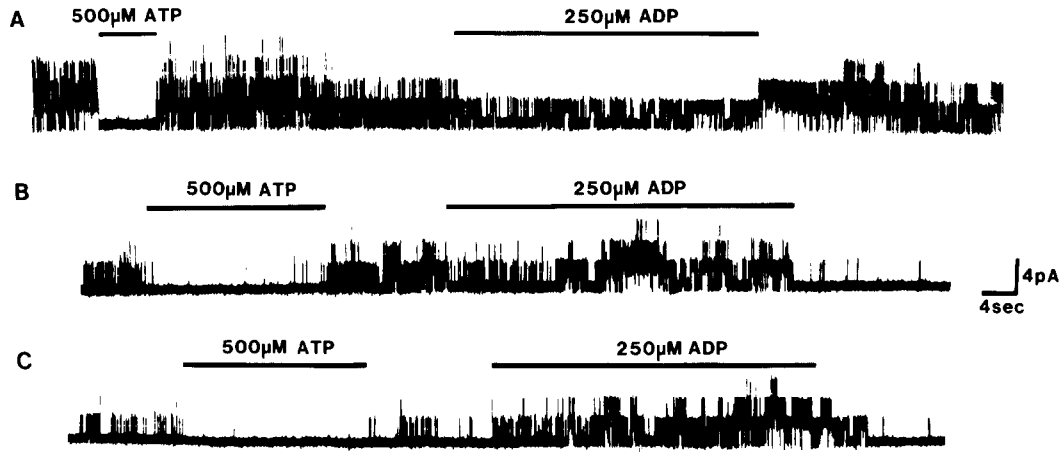

Fig.2. Cell-attached membrane patch current recordings all obtained from the same RINm5F cell after permeabilization. All details as in fig.1A–C are consecutive traces. There is a general run-down of channel activity throughout this experiment and while the effects of ATP are qualitatively the same in all 3 traces the effect of ADP changes from a simple inhibition in A to a biphasic inhibition-activation in B to a pure activation in C. The interval between A and B is 285 s and between B and C 205 s. All current traces were filtered at 500 Hz.

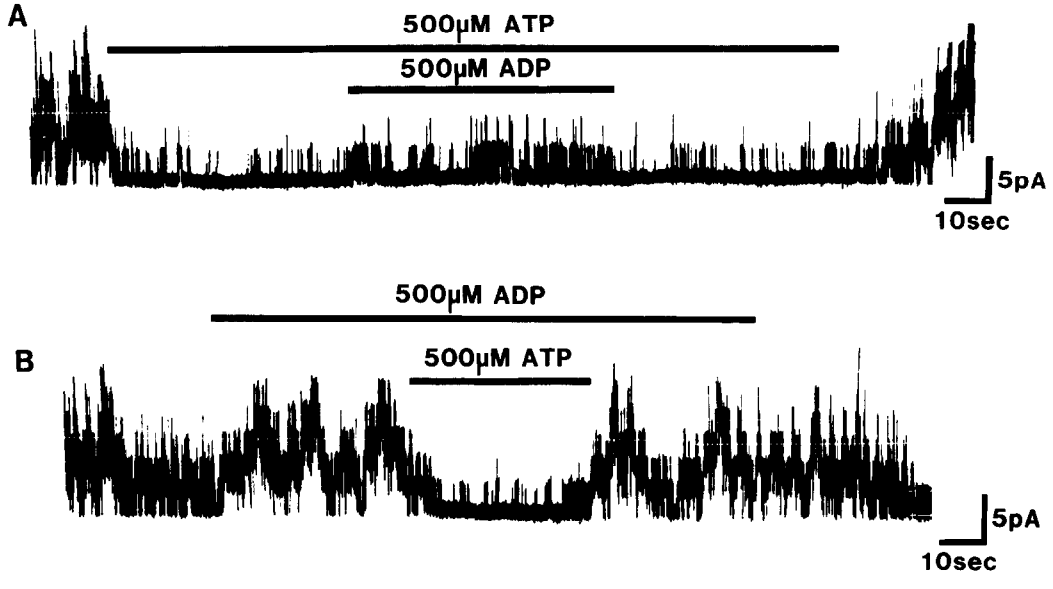

Fig.3. Continuous cell-attached membrane patch current recordings obtained from two separate RINm5F cells after permeabilization of plasma membrane outside isolated patch area. All details as in fig.1. Panel A shows the usual inhibitory effect of ATP, but when ADP is added to the ATP-containing solution there is reversible activation. Panel B shows the activating effect of ADP and demonstrates that ATP can reversibly close ADP-activated channels. The current traces were filtered at 500 Hz.

simple blocking effects were consistently observed in 6 separate cells. These effects were distinctly weaker than the blocking effects of the same concentrations of ATP investigated in the same experiments.

In contrast to the ADP action, AMP, cyclic AMP and adenosine had only weak and inconsistent effects. AMP (500 μM) was applied to 6 open

cells a total of 15 times and inhibition of the channels was observed on 6 occasions with activation seen 3 times while in the remaining 6 applications no inhibitory or activating effects were observed. Cyclic AMP (500 μM) evoked small activating effects in two cells but no clear effects in a third experiment. Adenosine (500 μM) was found to have no effect in 2 out of the four cells tested, but evoked slight activations in the two other experiments.

When ADP (500 μM) was added during a period of sustained ATP (500 μM)-evoked channel inhibition, activation occurred (fig.3A). This reversible ADP-evoked activation of ATP-inhibited K^+ channels was observed on all the eight occasions in 5 separate permeabilized cells where this protocol (fig.3A) was carried out. Conversely, ATP (500 μM) always closed ADP-activated channels in the three permeabilized cells where this experiment was done (fig.3B).

4. DISCUSSION

These results presented here demonstrate for the first time that ATP-inhibited K^+ channels can be activated by ADP in concentrations (100–500 μM) that are likely to be physiologically relevant [11,19]. It has recently been shown that ATP also has an activating effect on the K^+ channels in insulin-secreting cells which is, however, normally hidden by the more powerful inhibition [6,20]. The mechanism underlying the activating ATP effect is unknown, but could be similar to the one involved in the ADP action. The fact that the non-hydrolysable ADP analogue ADP-β-S was unable to cause activation might indicate a requirement for phosphorylation, but further work is needed concerning this point. The most important result is that ADP activates K^+ channels during ATP-evoked inhibition. This suggests that it is the ATP/ADP ratio rather than the ATP concentration that is relevant in determining the opening pattern of the K^+ channels. This would seem to offer a good explanation for the changes in channel activation occurring in insulin-secreting cells after metabolic stimulation [4–6] as the glucose- or glyceraldehyde-evoked reduction in K^+ channel activity evoked by an increase in intracellular ATP concentration would be enforced by the simultaneous decrease in ADP concentration.

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