

Regulation of a volume-sensitive anion channel in rat pancreatic β -cells by intracellular adenine nucleotides

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1. The patch-clamp technique in the whole-cell configuration was used to measure the effects of intracellular adenine nucleotides on activity of the volume-sensitive anion channel in single, isolated rat pancreatic β -cells.
2. In the absence of intracellular nucleotides, swelling of cells with a hypertonic pipette solution failed to activate the conductance. Addition of ATP over the range 2–10 mM maintaining the same degree of hypertonicity caused a progressive activation of the conductance. An increase in ATP produced a similar activation of the conductance in non-swollen cells, albeit with reduced current amplitudes.
3. Activation of the conductance was also observed in the presence of ATP γ S, adenylyl imidophosphate (AMP-PNP), ADP, diadenosine tetraphosphate and GTP γ S. Neither ADP nor GDP β S inhibited activation of the conductance by ATP.
4. It is concluded that activity of the β -cell volume-sensitive anion channel can be modulated by changes in intracellular concentrations of ATP within the physiological concentration range by a mechanism that does not require nucleotide hydrolysis. Activity of the channel does not appear to be modulated by a G protein-coupled mechanism.

The stimulation of insulin secretion by glucose requires metabolism of the sugar in the pancreatic β -cell and involves the generation of electrical activity that results from depolarization of the β -cell membrane. This depolarization is thought to result, at least in part, from the closure of K_{ATP} channels, which is itself mediated by an increase in ATP/ADP ratio resulting from glucose metabolism (see Ashcroft & Rorsman, 1989, for review).

An outwardly rectifying volume-sensitive anion-selective channel has recently been described in insulin-secreting cells (Best *et al.* 1995, 1996*b*; Kinard & Satin, 1995). Activation of this channel by cell swelling generates an inward current leading to depolarization and stimulated insulin release (Best *et al.* 1996*a*). Thus activation of this channel could also play a role in the modulation of β -cell electrical and secretory activity by glucose (Best, 1997). It is, however, unclear how activation of the anion channel is brought about. One possibility is that small changes in cell volume under normal physiological conditions could regulate the channel. For example, we have recently shown that glucose causes an increase in β -cell volume sufficient to activate the channel (Miley *et al.* 1997).

Although the volume-sensitive anion channel in the pancreatic β -cell appears to be distinct from that in other cell types, at least on the basis of the unusual halide selectivity sequence $Br > Cl > I$, activity of the β -cell channel is, in

common with other volume-sensitive anion channels, ATP dependent (Kinard & Satin, 1995; Best *et al.* 1996*b*). Thus in view of the changes in ATP and ADP levels thought to occur in glucose-stimulated β -cells (Detimary *et al.* 1996), an additional possibility is that activity of the channel could be modulated by changes in intracellular nucleotide concentrations. The present study demonstrates that the volume-sensitive anion channel in rat pancreatic β -cells is progressively activated by ATP over a range of concentrations encompassing those that would be expected to occur under physiological conditions. Activation of the channel can also be evoked by a range of other adenine and guanine nucleotides.

METHODS

Islet and β -cell preparation

Sprague–Dawley rats (300–400 g) of either sex were killed by stunning and cervical dislocation. Pancreatic islets were isolated by collagenase digestion (Lacy & Kostianovsky, 1965) and dispersed into single cells and small clusters by a 5–10 min incubation in a Ca^{2+} -free medium containing (mM): 135 NaCl, 5 KCl, 1 $MgSO_4$, 10 Hepes-NaOH and 1 EGTA; pH 7.4. The cells were then resuspended in Hepes-buffered RPMI medium (Gibco), plated into 30 mm diameter polystyrene dishes and cultured for 3–10 days in humidified air at 37 °C. Single β -cells, identified by their size and characteristic granular appearance, were used in all cases in order to avoid contaminating currents from adjacent electrically coupled cells.

Patch-clamp experiments

Cells were superfused at a rate of 4 ml min^{-1} with a medium consisting of (mM): 135 NaCl, 5 KCl, 1 NaH_2PO_4 , 1 MgSO_4 , 1.2 CaCl_2 , 4 glucose and 10 HEPES-NaOH (pH 7.4, 285 mosmol l^{-1}). Whole-cell current recordings from single β -cells were measured using a List EP-7 amplifier (List Electronic, Darmstadt, Germany) essentially as described previously (Best *et al.* 1996b). Cells were held at -30 mV and subjected to 500 ms pulses from -100 to $+100 \text{ mV}$ in 20 mV increments using pCLAMP software (Axon Instruments). Recordings were filtered at 3 kHz, sampled at 1 kHz and stored on computer disk. Current amplitudes were measured 20 ms after the start of the voltage pulse. Whole-cell capacitance, calculated by 'nulling' the capacitance transients, was 10–15 pF. Two basic intracellular (pipette) solutions were used. A hypertonic solution (305 mosmol l^{-1}) designed to induce cell swelling consisted of (mM): 30 CsCl, 1 MgSO_4 , 0.5 EGTA, 220 mannitol and 10 HEPES-NaOH (pH 7.2). A hypotonic solution (265 mosmol l^{-1}), similar in composition to the above but containing 180 mM mannitol, was used in experiments where cell swelling was avoided. In both cases, the addition of nucleotides was made by substitution of mannitol in order to maintain osmolarity of the solution. The latter was measured by freezing-point depression. Data are presented as

means \pm S.E.M. and statistical significance was ascribed by a two-tailed Student's unpaired *t* test.

Materials

ATP and ADP were supplied by Calbiochem, ATP γ S, GTP γ S and GDP β S by Boehringer and diadenosine tetraphosphate and adenylyl imidophosphate (AMP-PNP) by the Sigma Chemical Company.

RESULTS

Figure 1 shows the effects of increasing concentrations of Mg^{2+} -ATP on activity of the outwardly rectifying anion conductance in rat β -cells swollen by a hypertonic pipette solution. A progressive increase in the amplitude of both inward and outward currents was observed with increasing concentrations of ATP in the range 2–10 mM whilst maintaining the osmolarity of the pipette solution at 305 mosmol l^{-1} . No significant activation of the conductance in rat β -cells was seen with a low concentration (0.2 mM) of ATP (not shown). Figure 1*Ab* also shows a typical

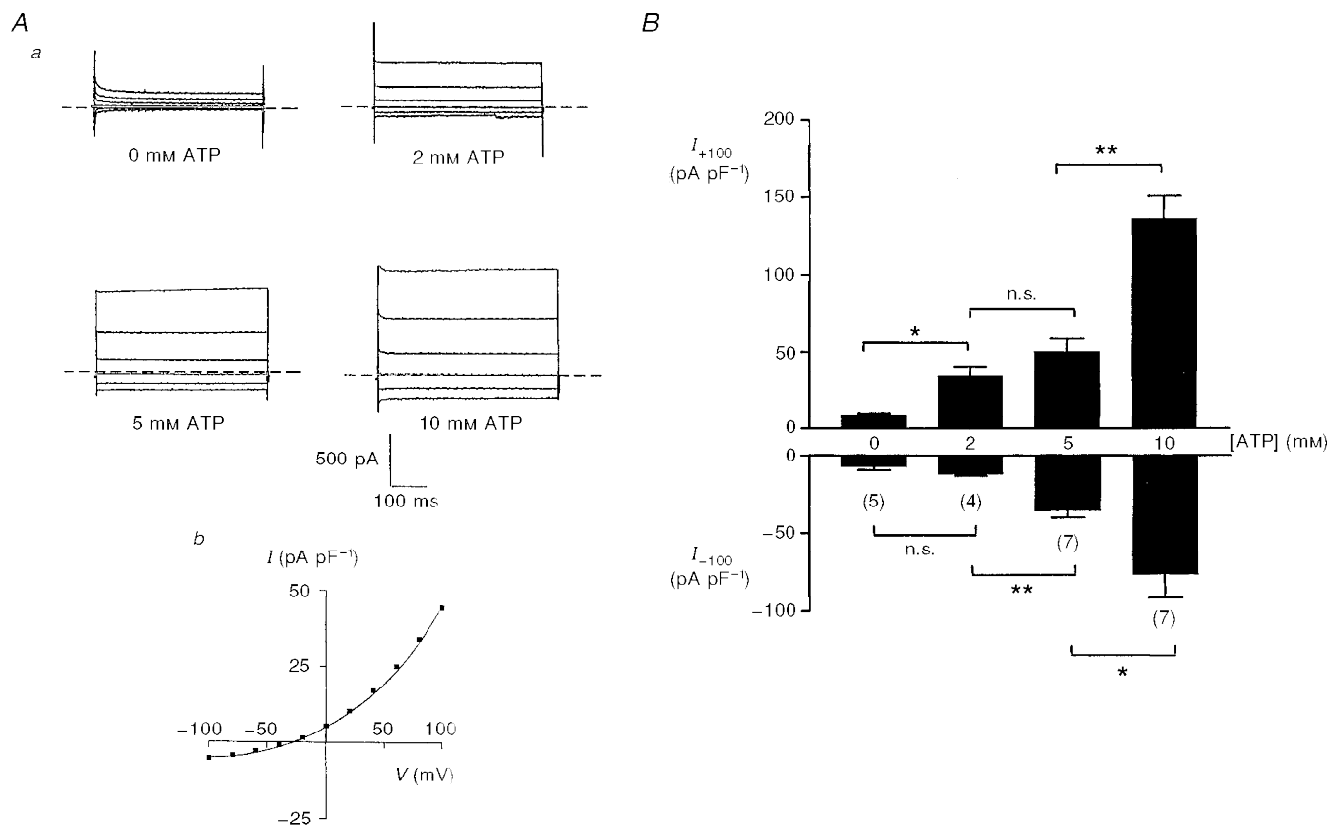
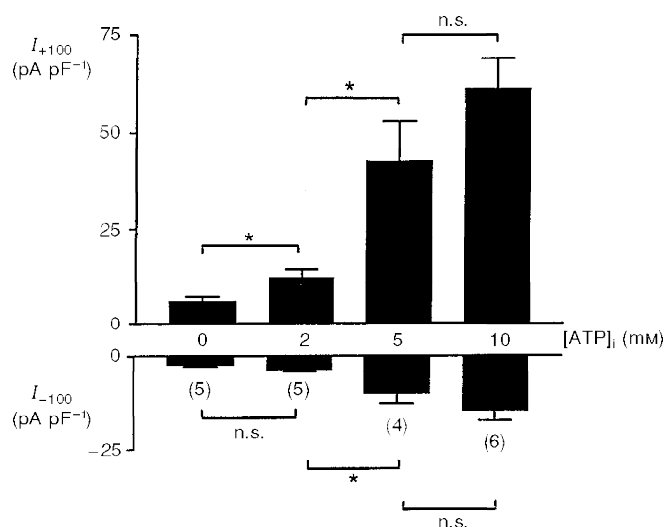


Figure 1. Effect of ATP concentration on the volume-sensitive anion conductance in swollen rat β -cells

Aa, typical current profiles with different concentrations of intracellular Mg^{2+} -ATP. Cells were held at -30 mV and subjected to 500 ms voltage pulses from -100 to $+100 \text{ mV}$. The pipette solutions were maintained at constant hypertonicity (305 mosmol l^{-1}) in order to induce cell swelling. *Ab*, current-voltage (*I-V*) plot for a typical cell dialysed with a pipette solution containing 2 mM ATP. The reversal potential for this cell was -31 mV . *B*, outward (I_{+100}) and inward (I_{-100}) current densities in response to voltage pulses of $+100$ and -100 mV , respectively: effects of increasing concentrations of Mg^{2+} -ATP. All values represent maximum currents attained in each cell. The values in parentheses represent the number of cells for each treatment. * $P < 0.05$, ** $P < 0.01$ between the groups as shown; n.s., $P > 0.05$.

Figure 2. Effect of ATP concentration on the volume-sensitive anion conductance in non-swollen rat β -cells

Outward (I_{+100}) and inward (I_{-100}) current densities in response to voltage pulses of +100 and -100 mV, respectively: effects of increasing concentrations of Mg^{2+} -ATP. The pipette solutions were maintained slightly hypotonic (265 mosmol l^{-1}) in order to prevent cell swelling. All values represent maximum currents attained in each cell. The values in parentheses represent the number of cells for each treatment. * $P < 0.05$, between the groups as shown; n.s., $P > 0.05$.



current-voltage plot for a cell dialysed with 2 mM ATP. The reversal potential of the conductance in this cell was -31 mV. The deviation of this value from the calculated E_{Cl} of -40 mV probably reflects the significant cation permeability of the channel (Best *et al.* 1996b). In order to examine the influence of added Mg^{2+} on activity of the conductance, control experiments were performed with $MgSO_4$ added to the pipette solution. With a pipette solution containing 10 mM $MgSO_4$ and no ATP, the current densities in response to voltage pulses of +100 and -100 mV were 10.9 ± 0.7 and -11.5 ± 2.0 pA pF⁻¹ ($n = 6$), respectively. In the presence of 2 mM ATP, addition of 10 mM $MgSO_4$ to the pipette solution markedly inhibited the outward currents, the corresponding current densities being 12.6 ± 1.0 and -16.3 ± 3.4 pA pF⁻¹ ($n = 6$).

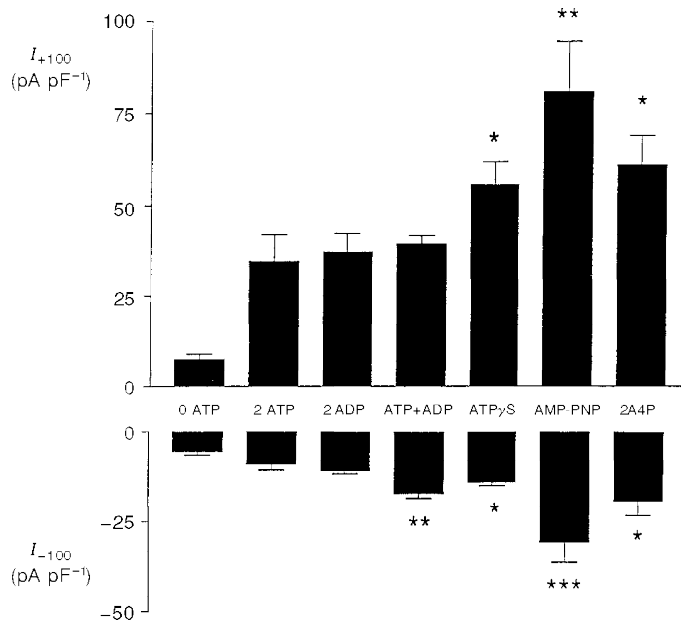
In cells dialysed with a slightly hypotonic pipette solution to prevent cell swelling, ATP caused a similar concentration-

dependent activation of channel activity (Fig. 2). Note, however, that under non-swollen conditions, the amplitudes of the volume-sensitive currents were, in general, less than the corresponding values in swollen cells. This effect of cell swelling was statistically significant in the presence of 2 mM ($P < 0.02$) and 10 mM ($P < 0.05$) ATP. The amplitude of the outward current measured at +100 mV showed linear correlation with concentration of ATP, the correlation coefficients being 0.96 and 0.95 in swollen and non-swollen cells, respectively.

Since ATP and ADP have been shown to have opposite effects on activity of the K_{ATP} channel (Kakei *et al.* 1986), we investigated the effects of ADP on activity of the volume-sensitive anion channel. As shown in Fig. 3, 2 mM ADP supported activation of the channel to the same extent as an equivalent concentration of ATP. ADP also failed to antagonize the stimulatory effect of ATP on channel

Figure 3. Activation of volume-sensitive anion conductance by adenosine nucleotides

Outward (I_{+100}) and inward (I_{-100}) current densities in response to voltage pulses of +100 and -100 mV, respectively: effects of various adenine nucleotides (all 2 mM; 2A4P, diadenosine tetraphosphate). The pipette solutions were maintained at constant hypertonicity (305 mosmol l^{-1}) in order to induce cell swelling. All values represent maximum currents attained in each cell. Each bar represents the mean \pm s.e.m. of 5-7 separate determinations. The asterisks denote significant differences from 2 ATP (column 2): * $P < 0.05$, ** $P < 0.02$, *** $P < 0.01$.



activity (Fig. 3). In order to assess whether the activation produced by ATP involved hydrolysis of the nucleotide, the non-hydrolysable analogues ATP γ S and AMP-PNP were also used. As shown in Fig. 3, both of these analogues at a concentration of 2 mM were effective in activating the conductance. Figure 3 also shows that channel activation could be induced by diadenosine tetraphosphate, levels of which have been reported to be sensitive to glucose in the pancreatic β -cell (Ripoll *et al.* 1996). The stimulatory effects of ATP γ S, AMP-PNP and diadenosine tetraphosphate on both outward and inward currents were significantly greater than an equivalent concentration of ATP ($P < 0.05$ or less).

It has been reported that a volume-sensitive anion channel in bovine chromaffin cells can be activated and inactivated, respectively, by the non-hydrolysable guanine nucleotides GTP γ S and GDP β S (Doroshenko *et al.* 1991; Voets *et al.* 1998). Furthermore, changes in guanine nucleotide concentrations have been demonstrated in glucose-stimulated β -cells (Detimary *et al.* 1996). The final series of experiments therefore examined the effects of guanine nucleotides on activity of the volume-sensitive anion channel. In the presence of 2 mM ATP, the non-hydrolysable GTP analogue GTP γ S (0.1 mM) failed to cause further activation of the conductance, whether in swollen cells (Fig. 4) or non-swollen cells (not shown). The inclusion of GDP β S (2 mM) in the pipette solution failed to inhibit the conductance, but caused a significant ($P < 0.05$) increase in amplitude of the outward current (Fig. 4). In swollen cells, the substitution of 2 mM ATP by 2 mM GTP γ S resulted in activation of the conductance, the amplitude of the outward current being significantly ($P < 0.05$) greater than with the equivalent concentration of ATP (Fig. 4).

DISCUSSION

An increase in intracellular ATP concentration is thought to be an important physiological modulator of β -cell electrical and secretory activity during stimulation by nutrients, by regulating activity of the K_{ATP} channel (Ashcroft & Rorsman, 1989). The results of the present study suggest

that changes in intracellular ATP concentration could also affect activity of the volume-sensitive anion channel. It has previously been demonstrated that swelling-induced activation of this channel requires ATP (Kinard & Satin, 1995; Best *et al.* 1996b). However, the present study demonstrates that a progressive increase in channel activity was apparent over a range of ATP concentrations (2–10 mM) that encompasses the expected levels of the nucleotide in an intact β -cell. Due to the fact that channel activation was still not apparently maximal at a high concentration (10 mM) of ATP, we have not formally estimated the half-maximally effective concentration of the nucleotide. However, this value would appear to be at least an order of magnitude higher than that of 0.3 mM, which has been reported to cause half-maximal inhibition of the K_{ATP} channel in whole-cell recordings from β -cells (Panten *et al.* 1989). The finding that addition of $MgSO_4$ to the intracellular solution failed to activate the conductance indicates that the stimulatory action of ATP- Mg^{2+} was not the result of increased $[Mg^{2+}]$. Indeed, in the presence of ATP, addition of a high concentration of Mg^{2+} was found to reduce the amplitude of the outward currents markedly. This observation is consistent with previous reports of inhibition of volume-sensitive anion channels by Mg^{2+} (Anderson *et al.* 1995).

The finding that ADP antagonizes the inhibition by ATP of K_{ATP} channel activity has led to the suggestion that the ATP/ADP ratio may be of greater importance than absolute concentration of ATP in the regulation of this channel (Kakei *et al.* 1986). However, in the case of the volume-sensitive anion channel, it appears unlikely that the channel is modulated by the ATP/ADP ratio, since both nucleotides at a concentration of 2 mM were found to cause channel activation to a similar extent, whilst ADP failed to reverse activation of the channel by ATP.

The activation of anion channel activity in swollen β -cells by ATP could also be demonstrated in non-swollen cells. This implies that the channel could be active under non-stimulated conditions, a suggestion consistent with the observation that channel inhibition by 4,4'-dithiocyanato-stilbene-2,2'-disulphonic acid leads to hyperpolarization of

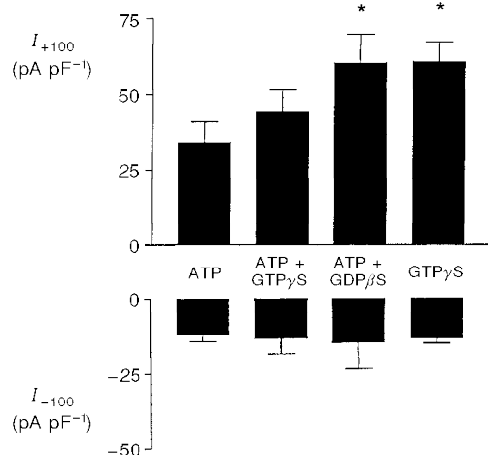


Figure 4. Effect of GTP γ S and GDP β S on the volume-sensitive anion conductance in rat β -cells

Outward (I_{+100}) and inward (I_{-100}) current densities in response to voltage pulses of +100 and -100 mV, respectively: effects of 2 mM ATP, 2 mM ATP + 0.1 mM GTP γ S, 2 mM ATP + 2 mM GDP β S and 2 mM GTP γ S alone. The pipette solutions were maintained at constant hypertonicity (305 mosmol l⁻¹) in order to induce cell swelling. All values represent maximum currents attained in each cell. Each bar represents the mean \pm s.e.m. of 4–5 separate determinations.

* $P < 0.05$ compared with 2 mM ATP alone (column 1).

the cell in the presence of a sub-stimulatory concentration of glucose (Best *et al.* 1996*a*). Furthermore, this finding suggests that changes in intracellular ATP concentration within the physiological range could be a primary signal in channel activation in the absence of cell swelling, or could modulate channel activation induced by swelling or some additional mechanism.

The ability of raised concentrations of ATP to potentiate activity of the volume-sensitive anion channel did not depend upon hydrolysis of the nucleotide. This is consistent with findings in some, but not all, cell types (see Okada, 1998, for review) and suggests that activation of the β -cell channel by ATP is not the result of phosphorylation of the channel protein. Thus it is possible that the channel may have a nucleotide binding site, which can bind a range of nucleotides and modulate channel activity by an allosteric mechanism. The finding that channel activation was also observed in the presence of diadenosine tetraphosphate and GTP γ S suggests that this putative binding site is non-selective and discriminates poorly between adenine and guanidine nucleotides. Our findings that channel activity could not be activated by a low concentration of GTP γ S, nor inhibited by GDP β S, suggest that unlike the volume-sensitive anion channel in bovine chromaffin and endothelial cells (Doroshenko *et al.* 1991; Voets *et al.* 1998), the anion channel in pancreatic β -cells is not modulated by a G protein-coupled mechanism.

In conclusion, this study demonstrates that activity of the volume-sensitive anion channel in rat pancreatic β -cells can be modulated in a concentration-dependent manner by intracellular ATP within the expected physiological range. This effect does not require ATP hydrolysis and was observed with other adenine and guanine nucleotides, and could thus represent activation of the channel via an allosteric nucleotide binding site. Changes in intracellular [ATP] could therefore be a physiological modulator of channel activity in the pancreatic β -cell.

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