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Effects of extracellular adenine nucleotides on the electrical, ionic and secretory events in mouse pancreatic β-cells

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1 The mechanisms whereby extracellular adenine nucleotides modulate pancreatic β-cell function were studied with mouse islets stimulated by 15 mM glucose.

2 Adenosine 5'-triphosphate (ATP) and adenosine 5'-diphosphate (ADP) (100 μM) inhibited insulin release, ^{45}Ca efflux and ^{86}Rb efflux from islet cells, and decreased electrical activity in β-cells. These changes were rapid but small and transient.

3 α,β-Methylene ADP caused a rapid and sustained inhibition of insulin release, ^{45}Ca efflux and ^{86}Rb efflux from islet cells. It also produced a slight hyperpolarization of the β-cell membrane, with sustained modification of the pattern but only transient decrease of the intensity of the electrical activity. In the absence of extracellular Ca^{2+}, α,β-methylene ADP increased ^{45}Ca and ^{86}Rb efflux without changing insulin release. Most effects of α,β-methylene ATP were qualitatively similar but quantitatively smaller than those of the ADP-analogue.

4 Adenylylimido-diphosphate (AMP-PNP) slightly increased ^{45}Ca and ^{86}Rb efflux and potentiated insulin release in the presence of extracellular Ca^{2+}. However, its effects on electrical activity in β-cells were qualitatively similar to those of the α,β-methylene analogues.

5 The small effects of ATP and ADP could result from their degradation into adenosine. α,β-Methylene ADP appears to increase K^+ permeability of the β-cell membrane and to produce a second, intracellular, effect which largely contributes to the inhibition of insulin release. Another recognition site, with higher affinity for triphosphate derivatives, could mediate the small stimulatory effects of AMP-PNP.

Introduction

Since the pioneer work of Burnstock (1972), it has been widely accepted that micromolar concentrations of extracellular adenosine 5'-triphosphate (ATP) and other nucleotides can influence numerous biological processes by acting on specific membrane receptors (Burnstock & Brown, 1981; Gordon, 1986; White, 1988).

It has long been reported that high concentrations of exogenous ATP increase insulin release *in vitro* (R-Candela *et al.*, 1963; Sussman *et al.*, 1969; Loubatières *et al.*, 1972; Feldman & Jackson, 1974). However, little attention was paid to these observations until Loubatières-Mariani *et al.* (1979) suggested that membrane purinoceptors could control β-cell function. This proposal was based on the evidence that micromolar concentrations of purine nucleotides markedly potentiate glucose-induced insulin release from the perfused rat pancreas. Subsequent functional studies by the same laboratory showed that the purinoceptor present on rat β-cells is of the P_{2y}-type (Chapal & Loubatières-Mariani, 1981a, b; Bertrand *et al.*, 1987), according to the classification of Burnstock & Kennedy (1985).

Recently, it has been proposed that ATP released by nerve endings could act in synergism with acetylcholine to increase insulin secretion (Bertrand *et al.*, 1986). It has also been speculated that ATP, which is co-secreted with insulin (Leitner *et al.*, 1975), could exert a positive feedback control of insulin release (Gylfe & Hellman, 1987; Blachier & Malaisse, 1988; Loubatières-Mariani & Chapal, 1988).

In the present work, mouse islets were used to investigate the possible mechanisms whereby ATP,

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ADP and some structural analogues affect insulin release. We focused on the changes in ionic fluxes and membrane potential, as they are critically involved in the regulation of β-cell function (Henquin & Meissner, 1984; Matthews, 1985; Petersen & Findlay, 1987).

Methods

All experiments were performed with islets of fed female NMRI mice (25–30 g), killed by decapitation. For electrophysiological experiments, a piece of pancreas was fixed in a small perifusion chamber, and the membrane potential of single β-cells was continuously recorded with microelectrodes (Meissner & Schmelz, 1974). β-Cells were identified by the typical electrical activity that they display in the presence of 10–15 mM glucose. For all other experiments, islets were isolated after collagenase digestion of the pancreas. For measurement of the efflux of ^{45}Ca or ^{86}Rb (used as tracer for K), the islets were first loaded with the tracers for 90 min (Garrino & Henquin, 1988). They were then transferred to a dynamic system of perifusion permitting monitoring of the efflux (Henquin, 1979). During the experiments of ^{86}Rb efflux, a portion of each effluent fraction was drawn for measurement of immunoreactive insulin; rat insulin was used as a standard (Henquin & Lambert, 1975).

The medium used had the following ionic composition (mM): NaCl 120, KCl 4.8, $CaCl_2$ 2.5, $MgCl_2$ 1.2, and $NaHCO_3$ 24. It was gassed with 94% O_2/6% CO_2 to maintain a pH 7.4, and was supplemented with 1 mg ml^{-1} bovine serum albumin fraction V (Boehringer, Mannheim, Germany), except for electrophysiological recordings. Ca^{2+}-free solutions were prepared by replacing $CaCl_2$ by $MgCl_2$.

The sodium salts of ATP and ADP were obtained from Boehringer (Mannheim, Germany). The sodium salt of α,β-methylene ADP, and the lithium salts of α,β-methylene ATP and adenylylimido-diphosphate (AMP-PNP) were purchased from Sigma Chemical Co (St Louis, MO, U.S.A.).

Electrophysiological experiments are illustrated by recordings, that are representative of the indicated number of experiments, performed with different mice. Certain changes in membrane potential were quantified manually on paper recordings. These measurements and results of ionic fluxes and insulin release are presented as means ± s.e.means.

Results

Effects of ATP and ADP

Addition of 100 μM ATP or ADP to a medium containing 15 mM glucose and 2.5 mM Ca^{2+} caused a small but rapid decrease in ^{86}Rb efflux and insulin release from perifused islets (Figure 1). These inhibitory effects were transient. At steady state, neither ^{86}Rb efflux nor insulin release was significantly

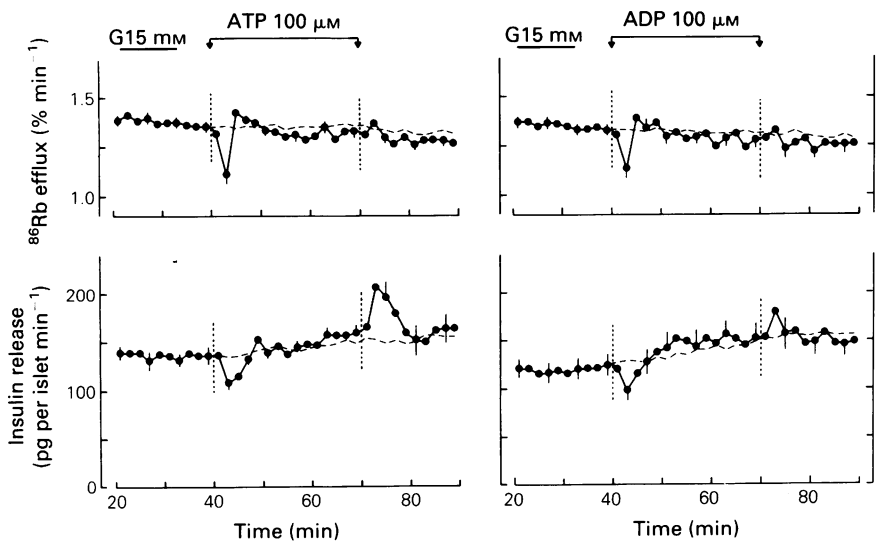

Figure 1 Effects of ATP and ADP on ^{86}Rb efflux and insulin release from mouse islets perifused with a medium containing 15 mM glucose (G). Control experiments without test substance are shown by broken lines. Values are means of 5 experiments; vertical lines show s.e.mean.

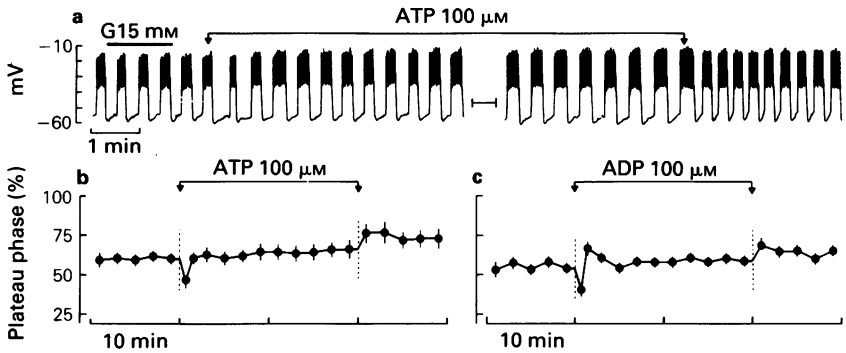

Figure 2 Effects of ATP and ADP on the membrane potential of mouse β-cells perifused with a medium containing 15 mM glucose (G). (a) This recording is representative of results obtained in 6 cells. The lower panels show the percentage of time spent at the plateau potential (with spike activity) by 6 cells stimulated with ATP (b) or ADP (c). Values are means and vertical lines show s.e.mean.

affected by the nucleotides. Removal of ATP was, however, consistently followed by a short-lived rebound of insulin release. This off-response was less marked and less consistently seen after withdrawal of ADP.

When mouse islets are perifused with 15 mM glucose, β-cells exhibit typical electrical activity consisting of repetitive slow waves of the membrane potential with bursts of rapid spikes superimposed on the plateau potential (Figure 2a). ATP consistently produced an initial decrease in this electrical activity, which was due to the shortening of one or two slow waves and the lengthening of one or two intervals. This was reflected by a decrease in the fraction of plateau phase during the first minute following addition of ATP (Figure 2b). Qualitatively and quantitatively similar changes were observed after addition of ADP to the medium (Figure 2c). These small changes are not artefacts simply produced by the change of solution, since they did not occur when the perifusion medium was alternately derived from two control solutions containing 15 mM glucose only (not shown). At steady state, the electrical activity remained marginally modified by ATP and ADP. The frequency of slow waves was slightly decreased because of a small lengthening of slow waves (Table 1). As these changes were rather small, the fraction of plateau phase was not significantly affected (Figure 2b and c). Removal of the nucleotides was, however, consistently followed by a small increase ($P < 0.05$) in electrical activity.

Table 1 Effects of ATP, ADP and analogues on the slow waves of membrane potential triggered by 15 mM glucose in mouse β-cells

Test substance (100 μM)	*Slow waves* (%)	*Intervals* (%)	*Frequency* (%)
ATP	120 ± 9	106 ± 9	90 ± 6
ADP	122 ± 6*	105 ± 7	87 ± 3*
α,β-methylene ADP	156 ± 8*	184 ± 13**	60 ± 3*
α,β-methylene ATP	165 ± 20*	135 ± 15	70 ± 8*
AMP-PNP	135 ± 6**	134 ± 24	76 ± 4**

In each experiment, slow waves and intervals were measured between 6 and 10 min after addition of the test substance. These values are expressed as a percentage of control values, measured in the same cell, during the last four min preceding the addition. For all cells, these control values were ($n = 27$): slow waves 13.0 ± 0.9 s; intervals 11.3 ± 0.9 s; frequency 2.7 ± 0.2 per min. AMP-PNP = adenylimido-diphosphate. Values are means ± s.e.mean for 5–6 cells. *$P < 0.05$, **$P < 0.01$ vs controls (by paired t test).

Effects of ATP and ADP analogues

In the presence of 15 mM glucose and 2.5 mM Ca^{2+}, α,β-methylene ADP caused a sustained and monophasic decrease in ^{45}Ca efflux and insulin release (Figure 3). The concomitant inhibition of ^{86}Rb efflux was biphasic: the initial marked fall was followed by a subsequent increase of the efflux rate to values slightly lower than in controls. In the absence of extracellular Ca, α,β-methylene ADP slightly increased ^{45}Ca efflux, more markedly accelerated ^{86}Rb efflux, and was without effect on basal insulin release (Figure 3). All these changes were reversible upon removal of α,β-methylene ADP. The analogue also modified glucose-induced electrical activity in β-cells. The membrane rapidly hyperpolarized by a few mV, while slow waves became shorter and intervals

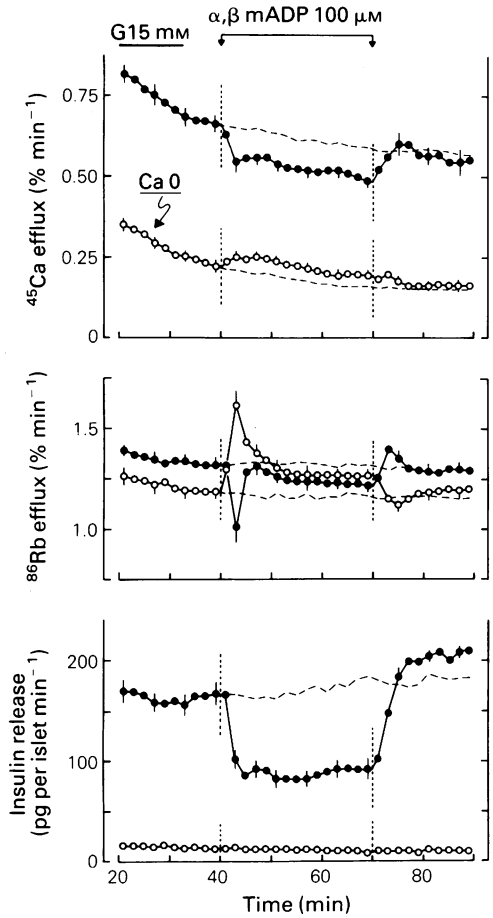

Figure 3 Effects of α,β-methylene ADP (α,β mADP) on ^{45}Ca or ^{86}Rb efflux and on insulin release from mouse islets perifused with a medium containing 15 mM glucose (G). The experiments were performed in the presence of 2.5 mM Ca^{2+} (●) or in the absence of added Ca^{2+} (see Methods) (○). Control experiments without addition of test substance are shown by broken lines. Values are means of 4–5 experiments; vertical lines show s.e.mean.

longer for 2–3 min (Figure 4a). At steady-state, the slope of the depolarization during the intervals was not so steep as in the absence of α,β-methylene ADP. However, the lengthening of the intervals was largely compensated for by a lengthening of the slow waves (Table 1). This explains why a significant inhibition of the plateau phase was only observed during the first minutes (Figure 4d). Yet, removal of the ADP analogue was followed by a consistent increase in activity with progressive resumption of the control pattern. The effects of α,β-methylene ADP were not prevented by blockade of α_2-adrenoceptors with 10 μM yohimbine (data not shown).

The changes in ^{45}Ca or ^{86}Rb efflux and in insulin release brought about by α,β-methylene ATP were qualitatively similar to those produced by α,β-methylene ADP (Figure 5), but were of smaller amplitude. The ATP analogue did not produce a rapid inhibition of the electrical activity (Figure 4b and e), but modified the characteristics of slow waves and intervals in a manner similar to that of the ADP analogue (Table 1).

Different results were obtained with AMP-PNP. This analogue slightly increased ^{45}Ca and ^{86}Rb efflux, and potentiated insulin release (Figure 6). It also caused a decrease in the frequency of slow waves, that was due to a lengthening of both slow waves and intervals (Table 1) and, hence, not accompanied by a significant change in overall electrical activity (Figure 4c and f). In the absence of extracellular Ca, 100 μM AMP-PNP had no effect on basal insulin release but still stimulated ^{86}Rb efflux; it had no consistent effect on ^{45}Ca efflux, a small initial increase being only occasionally observed (data not shown).

Since α,β-methylene ATP and AMP-PNP were used as lithium salts, control experiments were carried out to ascertain that lithium was not responsible for some of the effects. A low concentration (0.4–1 mM) of LiCl was without effect on insulin release, ^{86}Rb efflux and membrane potential (data not shown).

Discussion

The most unexpected observation of this study is that the response of mouse β-cells to ATP, ADP and their analogues is almost opposite to that of rat β-cells. Whereas all agents tested here increased insulin release from the perfused rat pancreas (Loubatières-Mariani *et al.*, 1979; Chapal & Loubatières-Mariani, 1981), only AMP-PNP produced a similar, small effect in mouse islets. The other agents rather caused a transient or sustained inhibition. This discrepancy is unlikely to be due to the isolation of the islets *per se*, since ATP, α,β-methylene ATP and α,β-methylene ADP have been found to increase insulin release in rat isolated islets (Petit *et al.*, 1987; Blachier & Malaisse, 1988). We are not aware of such a marked species-dependency of a tissue-response to purinoceptor stimulation. It is germane, however, to point out that activation of A_1-adenosine receptors causes a considerably smaller and shorter inhibition of insulin release in mouse islets (Petit *et al.*, 1988) than in rat islets (Campbell & Taylor, 1982; Bertrand *et al.*, 1989).

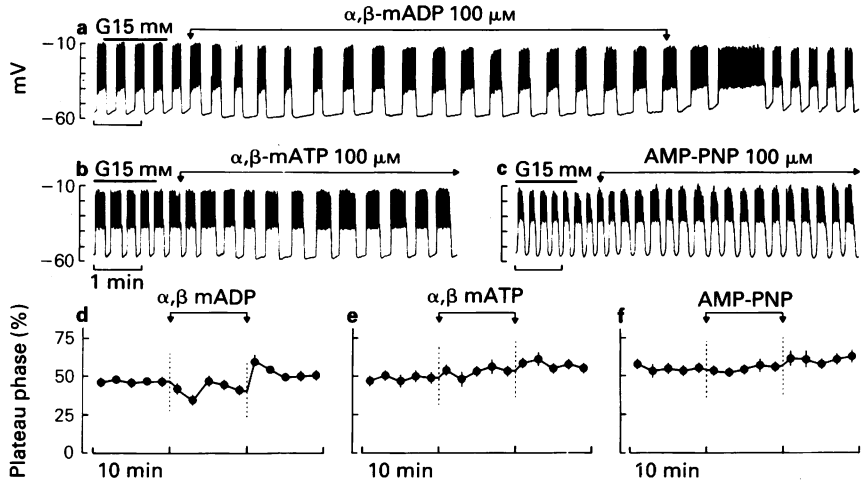

Figure 4 Effects of α,β-methylene ADP (α,β-MADP), α,β-methylene ATP (α,β mATP) and of adenylylimidodiphosphate (AMP-PNP) on the membrane potential of mouse β-cells perifused with a medium containing 15 mM glucose (G). Recordings (a), (b) and (c) are representative of results obtained in 5 cells each. The lower panels show the percentage of time spent at the plateau potential (with spike activity) in these cells. Values are means and vertical lines show s.e.mean.

A second unexpected finding is that the various putative agonists of P_2-purinoceptors that we used produced distinct effects. This may, however, be explained by their different stability.

The small effects of ATP and ADP on ^{86}Rb efflux, insulin release and β-cell membrane potential are strikingly similar to those produced by a low concentration (50 μM) of adenosine and by the adenosine analogue, N^6-(−)-phenylisopropyladenosine (Petit *et al.*, 1988). The most plausible interpretation, therefore, is that ATP and ADP are rapidly metabolized by ectonucleotidases (Gordon, 1986) and that adenosine acts on A_1-purinoceptors.

The sustained inhibitory effects of α,β-methylene ATP and α,β-methylene ADP, which cannot be metabolized to adenosine, strongly suggest the existence of a second inhibitory purinoceptor on mouse β-cells. As AMP-PNP did not mimic the effects of the methylene-analogues, one conservative hypothesis is that this second receptor has a preference for diphosphate derivatives, and recognizes α,β-methylene ATP only after partial hydrolysis. This latter would be consistent with the apparent fragility of ATP discussed above.

Finally, the small increase in insulin release produced by AMP-PNP could be best explained by a stimulatory purinoceptor with higher affinity for triphosphate derivatives. The fact that AMP-PNP did not cause the same rapid inhibitions as ATP and ADP might be explained if the analogue is not hydrolyzed to AMP (and then adenosine) because of the absence of ectopyrophosphatases on islet cells.

Obviously, our suggestion that mouse β-cells possess 3 recognition sites of purine derivatives will require further support from studies specifically designed to test this hypothesis. Except for the A_1-purinoceptor, for the existence of which separate evidence has been obtained (Petit *et al.*, 1988), it would be premature to name these sites according to the established nomenclature of purinoceptors (Burnstock & Kennedy, 1985; Gordon, 1986).

The causal links between glucose-induced electrical activity in β-cells and insulin release are well established (Henquin & Meissner, 1984). Adenine nucleotides partially dissociate these two events. A sustained inhibition of insulin release was produced by α,β-methylene ADP and α,β-methylene ATP, although the overall electrical activity was not or was only marginally decreased. A similar phenomenon was previously observed upon activation of α_2-adrenoceptors (Cook & Perara, 1982). It is striking that the reduction in slow wave frequency brought about by adrenaline was also associated with an increase in the duration of both slow waves and intervals. Moreover, the effects of adrenaline on ^{45}Ca and ^{86}Rb efflux were also similar to those produced by α,β-methylene ADP, at least in the presence of extracellular Ca^{2+} (Tamagawa & Henquin, 1983). Since the effects of the nucleotide persisted in the presence of yohimbine, one can exclude the hypothesis that it worked through α_2-adrenoceptors. The emerging conclusion is that α,β-methylene ADP, like adrenaline (through α_2-adrenoceptors), triggers an intracellular event that contributes to the inhibi-

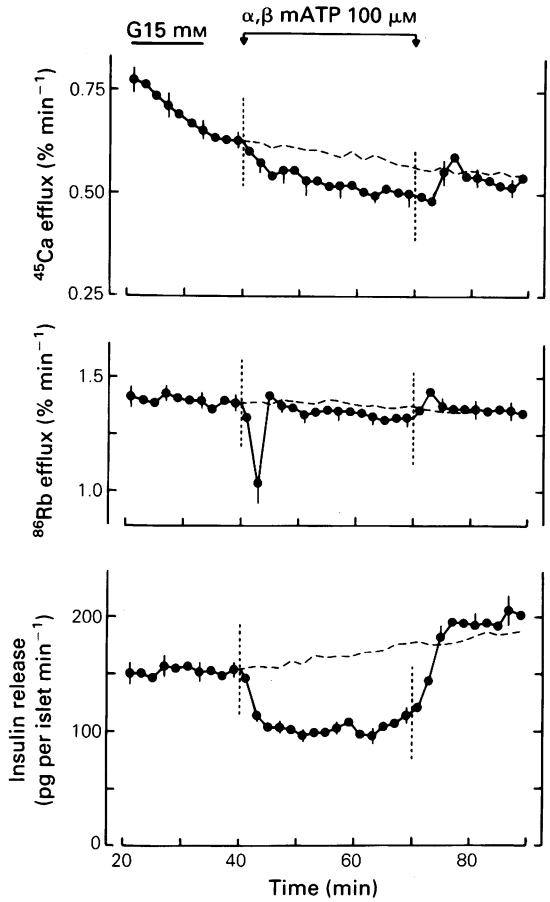

Figure 5 Effects of α,β-methylene ATP (α,β mATP) on ^{45}Ca or ^{86}Rb efflux and on insulin release from mouse islets perifused with a medium containing 15 mM glucose (G). The experiments were performed in the presence of 2.5 mM Ca^{2+}. Control experiments without addition of test substance are shown by broken lines. Values are means of 4–5 experiments; vertical lines show s.e.mean.

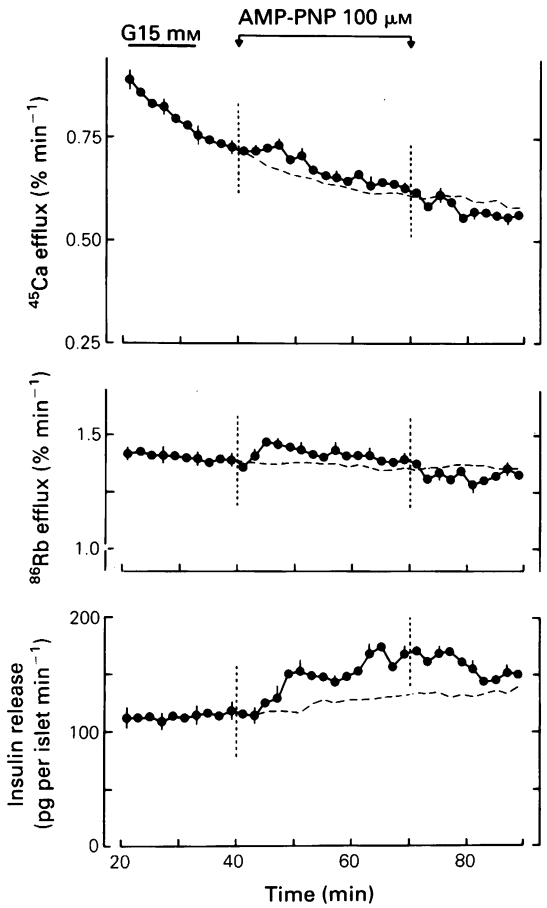

Figure 6 Effects of adenylylimido-diphosphate (AMP-PNP) on ^{45}Ca or ^{86}Rb efflux and on insulin release from mouse islets perifused with a medium containing 15 mM glucose (G). The experiments were performed in the presence of 2.5 mM Ca^{2+}. Control experiments without addition of test substance are shown by broken lines. Values are means of 4–5 experiments, vertical lines show s.e.mean.

tion of insulin release. Consistent with this conclusion is the observation that AMP-PNP affected electrical activity in a way qualitatively similar to that of α,β-methylene analogues, but increased insulin release. Evidence is indeed accumulating, which suggests that the membrane potential controls the triggering signal (Ca^{2+} influx), but that the cell response (insulin release) may be independently amplified or attenuated (Henquin *et al.*, 1987).

One important difference between the effects of α,β-methylene ADP and adrenaline is that the ADP analogue accelerated ^{86}Rb efflux in the absence of Ca^{2+}, whereas adrenaline decreased it (Tamagawa & Henquin, 1983). We consider this as good, albeit indirect, evidence that α,β-methylene ADP increases K^+ permeability in β-cells. The rapid fall in ^{86}Rb efflux that it produced in the presence of Ca^{2+} is not inconsistent with this idea. Thus, the initial hyperpolarization could result from the activation of one type of K^+ channel (ATP-sensitive?) by the nucleotide, and immediately attenuate the activation of other types of K^+ channels (voltage- and Ca^{2+}-dependent). The net effect is then a transient decrease in the efflux of ^{86}Rb. On the other hand, when Ca^{2+} influx does not occur (in Ca^{2+}-free solutions), the genuine action of the nucleotide to activate Ca^{2+}-

independent K^+ channels may be expressed by an acceleration of ^{86}Rb efflux. Activation of P_{2y}-purinoceptors leads to an increase in K^+ permeability in certain other tissues (Gallacher, 1982; Gordon & Martin, 1983).

In some tissues, activation of P_2 purinoceptors causes mobilization of intracellular Ca^{2+} (Charest *et al.*, 1985; Dubyak, 1986). This appears to be the case also in mouse islets, since α,β-methylene ADP slightly accelerated ^{45}Ca efflux in the absence of extracellular Ca. A similar conclusion was reached previously from experiments using ob/ob mouse islets, in which, however, ATP analogues triggered a more marked but less sustained increase in ^{45}Ca efflux (Gylfe & Hellman, 1987). That no insulin release occurred simultaneously is not surprising given the small magnitude of this Ca mobilization. The relationship between insulin release and Ca mobilization is non-linear (Garcia *et al.*, 1988).

In conclusion, only artificial adenine nucleotides have marked and sustained effects on insulin release by mouse islets. If one assumes that ATP and ADP released by nerve terminals within the islets or secreted with insulin exert similar effects to those of exogenously added nucleotides, the previous speculation that ATP participates in a positive feed-back control of β-cells does not hold for the mouse. Ectonucleotidases are thought to play an important role in the modulation of certain nerve cells by degrading locally released ATP to adenosine (Richardson *et al.*, 1987). Further experiments are needed to determine whether this is also the case in β-cells.

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