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Difference in the potentiating effect of adenosine triphosphate and α,β-methylene ATP on the biphasic insulin response to glucose

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1 The effects of exogenous adenine nucleotides and structural analogues on the biphasic insulin response to an increase of glucose concentration in the physiological range (from 4.2 to 8.3 mM) were studied in the isolated perfused rat pancreas. Purinoceptor agonists were added either simultaneously or 15 min before increasing glucose.

2 ATP and ADP at 16.5 μM were ineffective *per se* in the presence of the non stimulatory glucose concentration (4.2 mM) but markedly potentiated the biphasic insulin response to glucose rise in both experimental protocols.

3 Two more stable analogues of ATP and ADP (adenylylimidodiphosphate and α,β-methylene ADP (α,β-MeADP)) at 16.5 μM behaved like the natural compounds: they were ineffective at a glucose concentration of 4.2 mM and potentiated both phases of insulin response to glucose rise.

4 α,β-MeATP added simultaneously with the high glucose concentration, markedly potentiated the first phase of insulin response to glucose rise but did not potentiate the second one. When α,β-MeATP infusion began 15 min before glucose rise, the biphasic response to glucose was not potentiated, in contrast to what occurred with ATP.

5 In the presence of α,β-MeATP, the ATP potentiating effect was unaffected.

6 It is concluded that ATP and ADP, via activation of β cell P_{2y} purinoceptors, potentiates the biphasic insulin response to an increase of glucose concentration. On the other hand, α,β-MeATP did not behave like natural and other structural analogues of ATP and ADP: this difference appears not to be the consequence of desensitization of β cell P_{2y} purinoceptors by α,β-MeATP.

Introduction

Previously we have demonstrated that exogenous adenosine tri and diphosphate (ATP and ADP) stimulated insulin secretion from rat pancreatic β cell, an effect mediated by P_2 purinoceptor activation (Loubatières-Mariani *et al.*, 1979; Chapal & Loubatières-Mariani, 1981).

P_2 purinoceptors have been subdivided into P_{2x} and P_{2y}. This subdivision is principally based on the rank order of potency of ATP and its structural analogues. Furthermore administration of α,β-MeATP is known to desensitize P_2 purinoceptors in various tissues; this effect would be specific for P_{2x} receptor according to Burnstock & Kennedy (1985). Recently on the basis of the agonist potency order of ATP and its structural analogues, we have provided evidence for a P_{2y} purinoceptor subtype on rat β cell (Bertrand *et al.*, 1987).

It has long been established that raising the concentration of glucose, the major physiological stimulator of the β cell, induced a biphasic pattern of insulin secretion (Cerasi & Luft, 1967; Grodsky *et al.*, 1968). The aim of the present work was to investigate, in the isolated rat pancreas, the effect of the P_2 purinoceptor activation on the biphasic insulin response to an increase in glucose concentration. For this purpose we tested and compared the effect of ATP, ADP and their more stable structural analogues: adenylylimidodiphosphate (AMP-PNP), α,β-methylene ADP (α,β-MeADP) and α,β-methylene ATP (α,β-MeATP), on the biphasic insulin response

to an increase in glucose concentration in the range of that occurring in physiological conditions, namely during food intake in rat (Strubbe & Steffens, 1975).

Methods

Our experiments were performed on male Wistar rats fed *ad libitum* and weighing 320 to 350 g. The surgical procedure for the isolated perfused pancreas of the rat has been described (Loubatières *et al.*, 1969). After anaesthesia with sodium pentobarbitone (60 mg kg^{-1}), the pancreas was totally isolated from all neighbouring tissues; it was perfused through its own arterial system with a Krebs-Ringer bicarbonate buffer containing 2 g l^{-1} pure bovine serum albumin (Fraction V) and glucose 4.2 mM. The Krebs buffer had the following ionic composition (mM): NaCl 108, KH_2PO_4 1.19, KCl 4.74, $CaCl_2$ 2.54, $MgSO_4.7H_2O$ 1.19, $NaHCO_3$ 18. A mixture of O_2 (95%) and CO_2 (5%) was continuously bubbled through this medium; the pH was $\simeq$7.35. The preparation was maintained at 37.5°C. Each organ was perfused at a constant pressure, the flow rate was about 2.5 ml min^{-1}. In all the experiments, a 30 min adaptation period was allowed before taking the first sample for insulin assay. A second sample was taken 15 min later. At time 60 min, the glucose concentration was raised from 4.2 to 8.3 mM, the stimulation period lasting 30 min. In one experimental protocol ATP, ADP or α,β-MeATP were added simultaneously with glucose rise and remained for 30 min. In another protocol, adenine nucleotides were added to the physiological medium 15 min before glucose rise and remained for 45 min. For each group of experiments, controls (glucose stimulation) were performed. Samples were collected during a period of 1 min.

The kinetics of insulin output rate were studied for controls and for each adenine nucleotide. Samples were taken every min for 5 min, then at times 8, 10, 15, 20, 30 min just after increasing glucose concentration. The area under the curve was calculated for each experiment, both for the first phase, i.e. for the first 5 min, by adding the insulin output rates (ng min^{-1}) and for the second phase during the subsequent 25 min by applying the linear interpolation method.

Insulin in the pancreatic effluent was assayed by the radioimmunological method B of Hales & Randle (1963), using the SB-INSI-1 kit from CEA (France). Purified rat insulin (kindly supplied by NOVO, Copenhagen, Denmark) was used as the reference standard, the biological activity of which was 19 μu ng^{-1}. The intra and interassay coefficients of variations were respectively 9 and 13.5%.

Data are given as mean $\pm$ standard error of the mean (s.e.mean). For each group of experiments, areas under curves were submitted to analysis of variance by the multiple comparison test (Zar, 1974).

ATP and adenylylimidodiphosphate (AMP-PNP) were obtained from Boehringer Mannheim; ADP, α,β-MeATP and α,β-MeADP were from Sigma Chemical Company. ATP, ADP and α,β-MeADP were sodium salts, AMP-PNP and α,β-MeATP were lithium salts. Albumin (Fraction V) was from Sigma Chemical Company.

Results

Effect of exogenous ATP and ADP on the biphasic insulin response to a glucose rise

As shown in Figure 1, raising the glucose concentration in the perfusate from 4.2 to 8.3 mM (controls) elicited the classical biphasic pattern of insulin release.

To study the effects of ATP and ADP, two protocols were used: ATP or ADP was applied either simultaneously with (Figure 1a) or 15 min before (Figure 1b) the rise of glucose concentration from 4.2 to 8.3 mM. Since previous studies had shown that these nucleotides elicited half-maximum stimulating effect on insulin release at 16.5 μM, this concentration was used (Loubatières-Mariani *et al.*, 1979).

ATP or ADP 16.5 μM, introduced simultaneously with glucose 8.3 mM, markedly enhanced both phases of insulin response (Figure 1a). The areas under the curves were for the first phase respectively 62.9 $\pm$ 7.6 and 79.9 $\pm$ 18.9 ng ($P < 0.01$) for ATP and ADP versus 18.0 $\pm$ 2.7 ng in controls and for the second phase 573.6 $\pm$ 89.6 and 545.6 $\pm$ 114.4 ng ($P < 0.05$) versus 237.1 $\pm$ 44.8 ng in controls.

As shown in Figure 1b, ATP and ADP, introduced 15 min before the glucose rise, had no effect on basal insulin output rate recorded with a non stimulatory glucose concentration (4.2 mM). When glucose concentration was raised to 8.3 mM, ATP and ADP (16.5 μM) considerably enhanced the biphasic insulin response. The areas under the curves were for the first phase respectively 72.1 $\pm$ 9.9 and 71.2 $\pm$ 8.6 ng ($P < 0.001$) versus 22.4 $\pm$ 6.2 ng in controls, and for the second phase 554.6 $\pm$ 102.7 ($P < 0.01$) and 706.8 $\pm$ 59.6 ng ($P < 0.001$) versus 230.6 $\pm$ 59.6 ng in controls. These values are comparable to those recorded when the nucleotides were applied simultaneously with glucose rise.

Increasing the concentration of ATP (1.65, 16.5 and 165 μM) showed that the potentiation of the insulin response to glucose by ATP was concentration-dependent (results not shown). However, it should be noted that ATP at the lowest

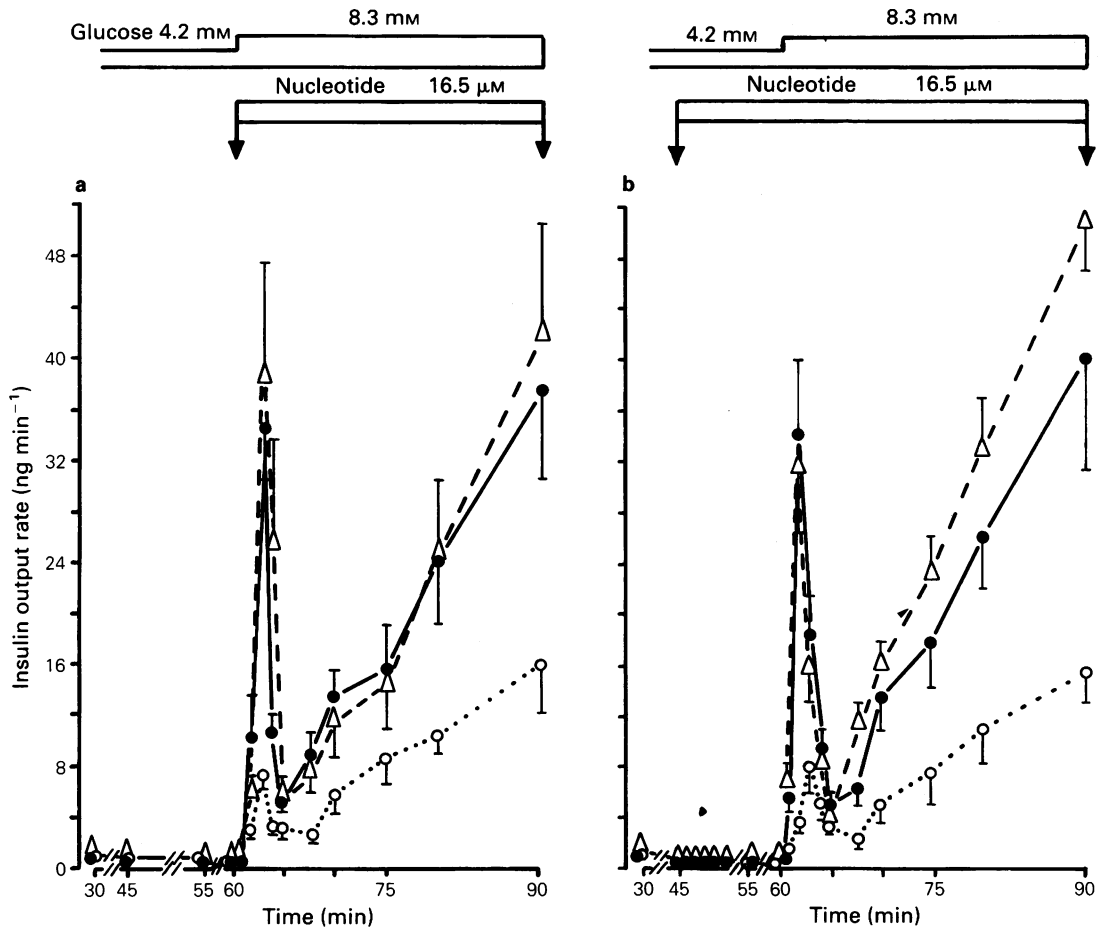

Figure 1 Effect of adenine nucleotides ATP (●) and ADP (△) (16.5 μM) on the biphasic insulin response to glucose rise; controls (○). (a) ATP and ADP were applied simultaneously with increased glucose. (b) ATP and ADP were applied 15 min before the glucose rise. Points represent the mean of 5–8 experiments and vertical lines the s.e.mean.

concentration (1.65 μM) significantly increased only the first phase.

Effect of two structural nucleotide analogues on the biphasic insulin response to a glucose rise (Figure 2)

In order to ascertain a potentiating effect of ATP and ADP *per se* and not after metabolic alteration, we studied the effects of AMP-PNP, a non phosphorylating ATP analogue and α,β-MeADP, a non hydrolysable methylene isostere of ADP (Yount, 1975). In these experiments we used the second protocol, the nucleotides were introduced 15 min before glucose increase.

While AMP-PNP and α,β-MeADP at 16.5 μM were ineffective in the presence of 4.2 mM glucose, they significantly increased the biphasic insulin response to glucose rise. The areas under the curves were respectively for the first phase 47.8 ± 10.1 and 59.1 ± 11.9 ($P < 0.05$) versus 17.9 ± 3.1 ng in controls, and for the second phase 460.1 ± 55.8 ($P < 0.05$) and 749.3 ± 97.4 ($P < 0.001$) versus 194.2 ± 27.2 ng in controls.

Effect of α,β-methylene ATP on the biphasic insulin response to a glucose rise (Figure 3)

As this substance is known to desensitize P_{2x} purinoceptors we used two experimental protocols.

α,β-MeATP (16.5 μM), when introduced simultaneously with the increase in glucose concentration from 4.2 to 8.3 mM (Figure 3a), induced a potentiation of the first phase of the insulin response to

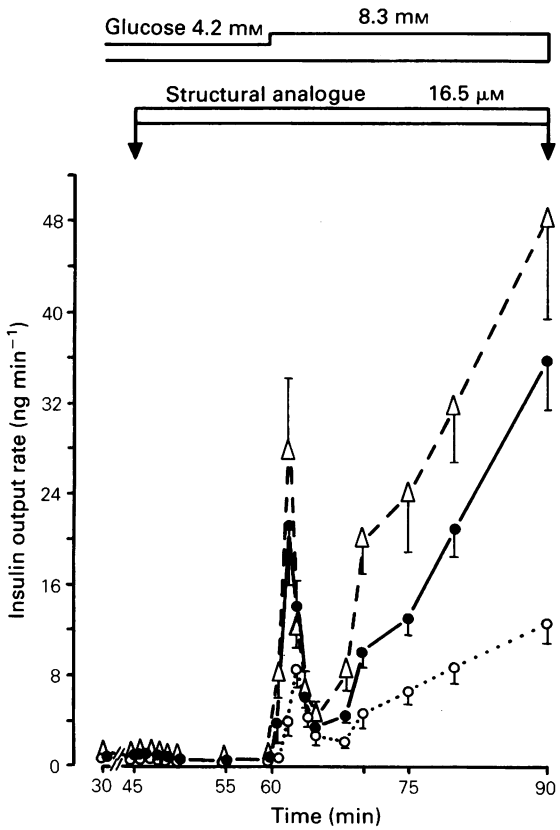

Figure 2 Effect of structural nucleotide analogues on biphasic insulin response to glucose rise. Adenylylimidodiphosphate (●); α,β-methylene ADP (△) and controls (○). Points represent the mean of 6–8 experiments and vertical lines the s.e.mean.

glucose, while the second phase was not significantly modified. It should be noted that in the presence of α,β-MeATP the response was delayed by about 3 min. This delay is explained by the interference of a vasoconstrictor effect of α,β-MeATP on the pancreatic vascular bed; this marked but transient effect has been described previously (Chapal & Loubatières-Mariani, 1983).

When α,β-MeATP was added 15 min before glucose rise (Figure 3b), the following results were obtained: (1) Unlike ATP, α,β-MeATP in the presence of a non stimulating glucose concentration induced a weak and transient but significant insulin secretion; the area under the curve was 15.0 ± 4.9 ng versus 3.2 ± 0.8 ng in controls ($P < 0.05$). (2) α,β-MeATP did not potentiate, as did ATP in the preceding experiments, the biphasic insulin response to glucose increase.

Effect of ATP on the biphasic insulin response to a glucose rise in presence of α,β-methylene ATP (Figure 4)

To determine whether the lack of effect of α,β-MeATP is due to a desensitization of P_2 purinoceptors of β cells, we have investigated whether the ATP effect was affected by the presence of this analogue.

To ensure an eventual desensitization we used a higher α,β-MeATP concentration (49.5 μM). As shown in Figure 4, ATP with and without α,β-MeATP elicited a comparable potentiating effect on the biphasic insulin response to glucose rise. The areas under the curves were respectively for the first phase 71.4 ± 5.3 and 69.5 ± 14.3 ng and for the second phase 438.7 ± 34.5 and 416.9 ± 84.7 ng in the absence or in the presence of α,β-MeATP. These values are comparable to those obtained in previous experiments with ATP (Figure 1a and 1b).

Discussion

The present results show that (1) ATP and ADP potentiate the biphasic insulin response to glucose; (2) α,β-MeATP behaves differently.

ATP and ADP are ineffective *per se* on insulin secretion in the presence of a substimulating glucose concentration and markedly potentiate the biphasic insulin response to a glucose increase in the physiological range (from 4.2 to 8.3 mM). Indeed, such an increase is close to that reported to occur in rat (Strubbe & Steffens, 1975) and man after a large carbohydrate meal (Müller & Weir, 1974). Similar results were obtained with two more stable structural analogues. Indeed, the non phosphorylating analogue of ATP, adenylylimidodiphosphate (AMP-PNP) potentiated as did ATP, both phases of insulin response to glucose rise, therefore excluding the involvement of ecto-ATPases for ATP action; on the other hand, the α,β-methylene isostere of ADP, resistant to hydrolysis to AMP and adenosine was as potent as ADP. So, the potentiating effect of ATP and ADP on the biphasic insulin response to a physiological glucose rise, appears to require neither hydrolysis of high energy phosphate bonds, nor the action of metabolic products such as AMP or adenosine. The potentiating action of ATP and ADP on the biphasic insulin response to glucose appears to be mediated through the P_2 purinoceptor previously characterized on the rat pancreatic β cell membrane (Loubatières-Mariani *et al.*, 1979; Chapal & Loubatières-Mariani, 1981).

α,β-MeATP potentiated the first but not the second phase, when it was introduced simultaneously with increased glucose. On the other hand,

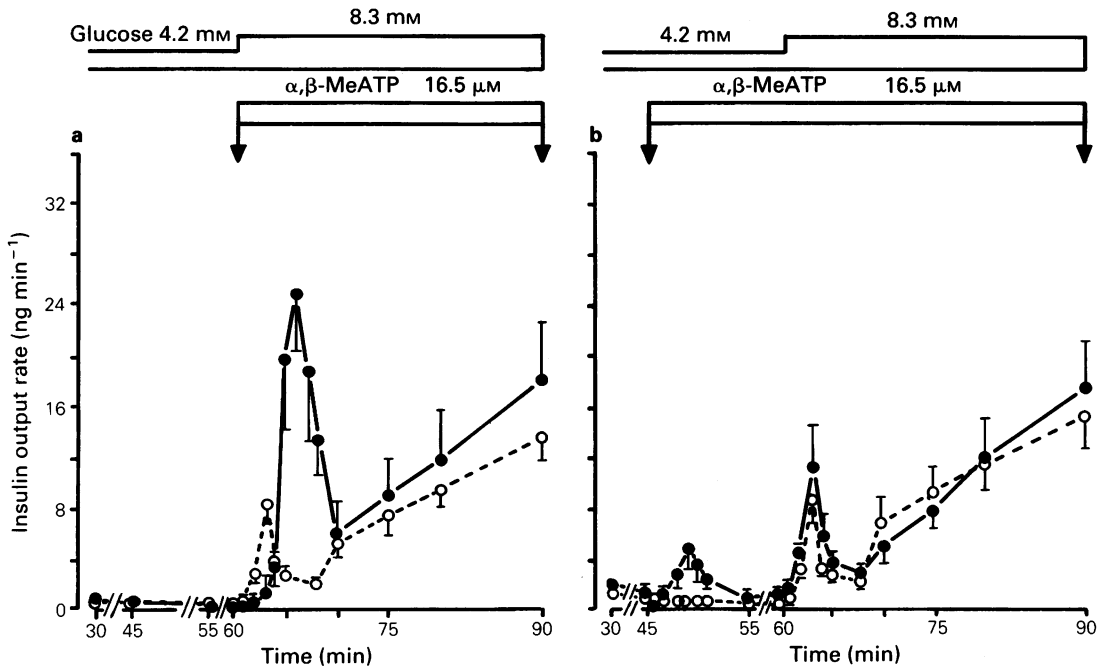

Figure 3 Effect of α,β-methylene ATP (α,β-MeATP) (16.5 μM) (●) on the biphasic insulin response to increased glucose. (a) α,β-MeATP was applied simultaneously with glucose rise. (b) α,β-MeATP was applied 15 min before glucose rise. Controls (○). Points represent the mean of 5–6 experiments and vertical lines the s.e.mean.

when α,β-MeATP was added 15 min before glucose rise, the biphasic response was not at all potentiated.

Thus there is a difference between the effects of α,β-MeATP and ATP on the biphasic insulin response to glucose increase. These results differ from those obtained when α,β-MeATP was applied 45 min after glucose stimulation (8.3 mM); in this case α,β-MeATP, like ATP, elicited a sustained insulin response (Chapal & Loubatières-Mariani, 1981). Since in both studies, α,β-MeATP induced a similar transient decrease in pancreatic outflow rate, the vasoconstrictor effect of this agent cannot explain the difference in the responses observed according to the experimental conditions concerning the glucose concentration. So, the previous glucose (substimulating or stimulating) concentration may play a role in the effects observed with α,β-MeATP. In this respect, the importance of the glucose concentration for the β-cell response to various stimulating agents must be recalled.

Tachyphylaxis has been shown to occur previously with α,β-MeATP on guinea-pig vas deferens (Meldrum & Burnstock, 1983) and cat colon (Hedlund *et al.*, 1983). This effect is generally the consequence of P_{2x} purinoceptor desensitization as it has been reported in such organs as mesenteric artery (Kügelgen & Starke, 1985; Muramatsu, 1986), vas deferens (Sneddon & Burnstock, 1984) and urinary bladder (Kasakov & Burnstock, 1983; Hoyle & Burnstock, 1985). However in our study since the ATP potentiating effect was unaffected in the presence of α,β-MeATP, the absence of the analogue potentiating effect is unlikely to be due to a desensitization of the P_2 purinoceptor which mediates ATP effect. We have previously characterized the P_2 purinoceptor of the rat β cell as a P_{2y} subtype on the basis of the agonist potency order of ATP and its structural analogues (Bertrand *et al.*, 1987). As it is known that α,β-MeATP selectively exerts a desensitization action at P_{2x} purinoceptors, our results provide further evidence for an ATP effect on rat β cell via the P_{2y} purinoceptor.

The discrepancy between ATP and α,β-MeATP remains to be explained. α,β-MeATP could act by an indirect action, e.g. via intramural nerves, as proposed by Moody & Burnstock (1982) in rat ileum. On the other hand, it might be suggested that the difference in the effect between α,β-MeATP and ATP could be due to the stimulation of the two different P_2 receptor subtypes. It is known that α,β-MeATP is more potent than ATP at P_{2x} receptors (Burnstock & Kennedy, 1985) and, in our experiments for the

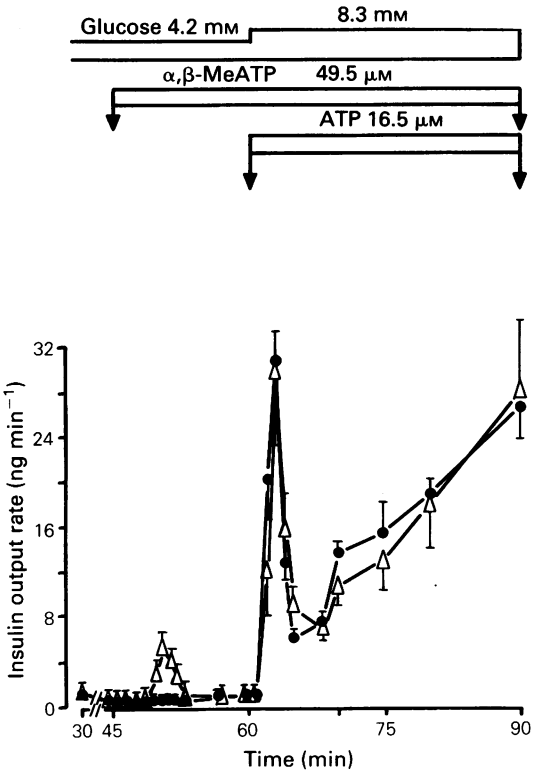

Figure 4 Effect of ATP (16.5 μM) on biphasic insulin response to glucose rise in absence (●) or in presence (△) of α,β-methylene ATP (49.5 μM). Points represent the mean of 4–5 experiments and vertical lines the s.e.mean.

same concentration (16.5 μM), α,β-MeATP, but not ATP, caused a small and transient increase of basal insulin release; at a ten fold higher concentration (165 μM), ATP elicited, only in some cases, a response in the same range (results not shown). However, further studies are needed to establish the existence of a P_{2x} receptor on β cells.

The potentiating effect of ATP and ADP on the biphasic insulin response to glucose rise might be of physiological relevance. Indeed, since ATP and ADP are present in the secretory granule and coreleased with insulin (Leitner *et al.*, 1975), they could exert a positive feedback control on the β cell during glucose stimulation. Furthermore as ATP strongly potentiates insulin release, particularly the first phase, it would be of interest to examine whether abnormalities in the P_2 purinoceptor of the β cell could be involved in the pathogenesis of non-insulin-dependent diabetes mellitus (NIDDM).

In conclusion, ATP and ADP potentiate the biphasic insulin response to a physiological glucose rise, an effect mediated by the P_{2y} purinoceptor of the β cell. On the other hand, α,β-MeATP has a different effect which cannot be explained by a desensitization of the P_{2y} purinoceptor of the β cell, but possibly implicates a P_{2x} purinoceptor.

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