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Evidence for two different P_2-purinoceptors on β cell and pancreatic vascular bed

[1]G. Bertrand, J. Chapal, M.M. Loubatières-Mariani & M. Roye

Faculté de Médecine, Laboratoire de Pharmacologie, UA 599 du CNRS, Institut de Biologie, Boulevard Henri IV, 34060 Montpellier Cedex, France

1 The effects of a 2-substituted analogue of adenosine 5'-triphosphate (ATP), 2-methylthioadenosine triphosphate (2-methylthio ATP) have been studied on insulin secretion and flow rate of the isolated pancreas of the rat, perfused in the presence of glucose (8.3 mM).

2 2-Methylthio ATP (16.5–1650 nM) increased insulin secretion in a biphasic and concentration-dependent manner; the kinetics were comparable to those previously obtained with ATP. A comparison of relative potency between ATP and 2-methylthio ATP showed that 2-methylthio ATP was 45 times more potent that ATP.

3 2-Methylthio ATP also provoked a transient decrease of the flow rate in a concentration-dependent manner but at concentrations (165–825 μM) about 1000 fold higher than those needed to increase insulin secretion. A comparison of relative potency between the natural derivative and 2-methylthio ATP showed that 2-methylthio ATP was only twice as potent as ATP.

4 These and other previous results (with phosphate-modified analogues of ATP) provide evidence for two different types of P_2-purinoceptors on endocrine cell and vessel cells of the pancreas. A P_{2Y} subtype, mediating an increase of insulin secretion, is present on the β cell of the pancreas. A P_{2X} subtype, mediating vasoconstriction, is present on the vascular bed of the rat pancreas.

Introduction

Adenosine 5'-triphosphate (ATP) and its derivatives play an important role in numerous physiological processes (Burnstock & Brown, 1981; Gordon, 1986). Pharmacological studies performed on the isolated perfused pancreas of the rat, demonstrated that insulin secretion was increased by ATP via a P_2-purinoceptor (Loubatières-Mariani *et al.*, 1979; Chapal & Loubatières-Mariani, 1981a) and pancreatic vascular bed was constricted by ATP at high concentration, also via a P_2-purinoceptor (Chapal & Loubatières-Mariani, 1983). However, the rank order of potency between ATP and its structural analogues modified on the polyphosphate chain was not the same for the two responses. Thus, the agonist potency order on insulin secretion was ATP > α,β-methylene ATP > AMP-PNP (adenylimido-diphosphate); β,γ-methylene ATP being inactive. On the decrease of flow rate the order was α,β-methylene ATP > β,γ-methylene ATP ≃ AMP-PNP > ATP. This finding suggested that two different types of P_2-purinoceptors were present on the pancreatic β cell and vascular bed (Loubatières-Mariani & Chapal, 1984).

[1]Author for Correspondence.

Recently a subdivision of P_2-purinoceptor into two types P_{2X} and P_{2Y} has been proposed by Burnstock & Kennedy (1985). This subdivision is largely based on the agonist potency order of structural analogues of ATP. The most potent agonists at the P_{2X} subtype are the phosphate-modified analogues of ATP. In contrast, 2-substituted analogues, such as 2-methylthio ATP are the most potent at the P_{2Y} subtype (Gordon, 1986).

The aim of the present work was to investigate the effects of 2-methylthio ATP, a more specific P_{2Y} agonist, on insulin secretion and flow rate in the isolated perfused pancreas of the rat. The comparison of these effects with those of ATP and those previously obtained with phosphate-modified analogues of ATP, provides evidence for the presence of a P_{2Y} on the β cell and a P_{2X} receptor on the vascular bed of rat pancreas.

Methods

Our experiments were performed on male Wistar rats fed *ad libitum* and weighing 330 to 360 g. The surgical procedure for the rat isolated perfused pancreas has

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been previously described (Loubatières *et al.*, 1969; Bertrand *et al.*, 1986). 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 8.3 mM. The Krebs buffer had the following 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 about 7.35. The preparation was maintained at 37.5°C. Each organ was perfused at a constant pressure: the pressure was selected so as to produce a flow rate of approximately 2.5 ml min^{-1} at the start of the experiments.

In all the experiments, a 30 min adaptation period was allowed before taking the first sample. A second sample was taken 15 min later, this one represented the reference sample. The Krebs solution supplemented with ATP or 2-methylthio ATP, was then perfused for 15 or 30 min. Samples were taken every min during the first 5 min, then at 7, 10, 15, and at 20 and 30 min when the drug was perfused for 30 min. The flow rate was measured during 1 min for each sample which was then immediately frozen for insulin radioimmunoassay. Insulin was assayed by the method of Hales & Randle (1963) using the SB-INSI-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 22.3 μu ng^{-1}. The intra- and inter-assay variations were respectively 9 and 13.5%.

Analysis of results

For the kinetics of insulin output rate and flow rate, the results for each point are expressed as changes in relation to the value at time 45 min taken as 100%.

Data are expressed as mean ± standard error of the mean (s.e.mean).

In order to establish the concentration-response curves for 2-methylthio ATP and ATP we used: (1) for insulin secretion, the increase of mean insulin output rate as a percentage. This value was obtained as follows: AUC/30 (AUC = area under the curve during the 30 min infusion). (2) for the flow rate, the mean of the values corresponding to the maximum drop in flow rate during the first 5 min in each experiment.

The values obtained were plotted as a function of the logarithm of 2-methylthio ATP or ATP concentrations.

Drugs

Adenosine-5'-triphosphate (ATP) was purchased from Boehringer Mannheim; 2-methylthioadenosine 5'-triphosphate (2-methylthio ATP) was supplied by Research Biochemicals Inc. Both were sodium salts.

Results

Effect of 2-methylthio ATP on insulin secretion; comparison with ATP

2-Methylthio ATP was studied at concentrations ranging from 16.5 to 16,500 nM. As shown in Figure 1, this 2-substituted analogue of ATP, induced a biphasic insulin response which was concentration-dependent in the range 16.5–1650 nM. There appeared an immediate first phase which culminated at the 2nd min and lasted 5 min; then the secretion rose again and remained high throughout the 2-methylthio ATP

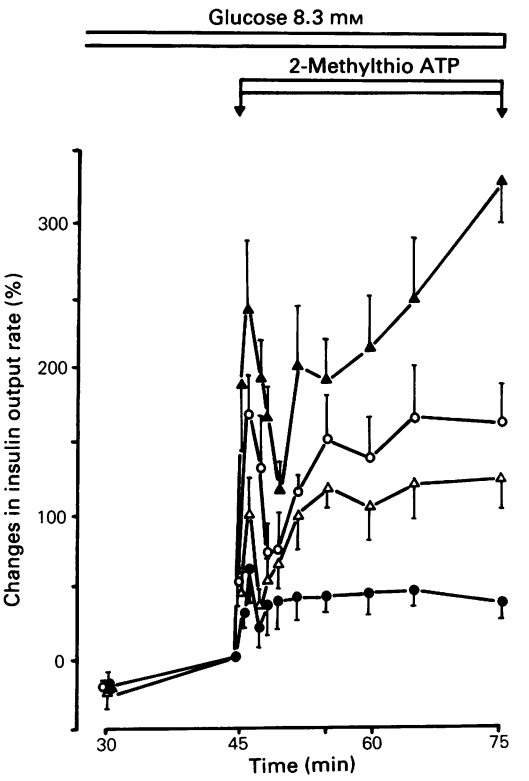

Figure 1 Effects of increasing concentrations of 2-methylthio ATP on insulin secretion from the isolated perfused pancreas of the rat: (●) 16.5 nM ($n = 5$), (△) 165 nM ($n = 5$), (○) 495 nM ($n = 4$), (▲) 1650 nM ($n = 5$). The insulin output rate (ng min^{-1}) at 45 min for each set of experiments was 21.38 ± 2.52; 26.21 ± 4.84; 22.42 ± 0.61 and 18.87 ± 6.06 respectively. Each point represents the mean with s.e.mean shown by vertical lines.

administration. When 2-methylthio ATP was used at 16,500 nM, insulin response was not significantly different from that obtained with 1650 nM (results not shown).

The concentration-response curves for 2-methylthio ATP and ATP are shown in Figure 2. A comparison of potency was performed by the parallel line assay method (Armitage, 1980). Linear regression analysis for the increase of 'mean insulin output rate' was carried out. The two concentration-response curves did not deviate significantly from parallelism. The potency of 2-methylthio ATP was 45 fold that of ATP with [20–90] for 95% confidence limits.

Effect of 2-methylthio ATP on pancreatic flow rate; comparison with ATP

2-Methylthio ATP at concentrations effective on insulin secretion, was ineffective on the flow rate (results not shown). In contrast, at higher concentrations (165, 495 and 825 μM) it induced an immediate and transient decrease of the pancreatic flow rate in a concentration-dependent manner (Figure 3). The maximum decrease was reached at the first min and, although the infusion lasted 15 min, the return up to the basal value occurred within 5 min. Decrease in pancreatic flow rate was also observed with ATP (Chapal & Loubatières-Mariani, 1983). In the present series of experiments, ATP was tested at the same concentrations as 2-methylthio ATP. The concentration-response curves for these two agonists are shown in Figure 4.

The relative potencies of the two nucleotides were compared by the parallel line assay method (Armitage, 1980). Linear regression analysis showed that the two concentration-response curves did not deviate significantly from parallelism. 2-Methylthio ATP was slightly more potent than ATP, only 2 fold with [1.8–2.9] for 95% confidence limits.

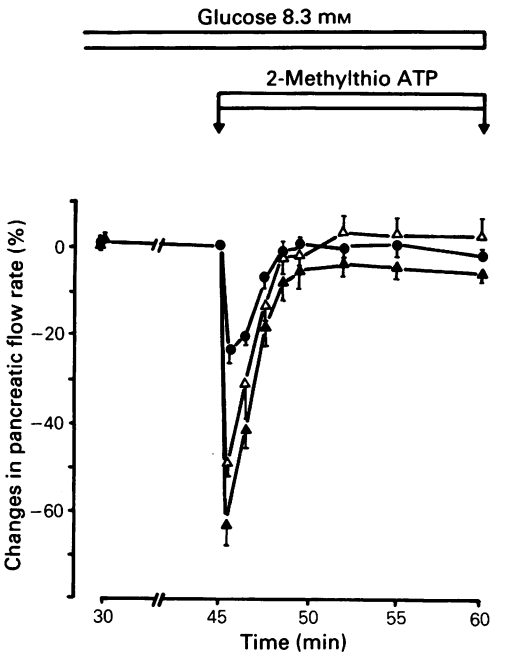

Figure 3 Effects of increasing concentrations of 2-methylthio ATP on pancreatic flow rate of the rat: (●) 165 μM ($n = 4$); (△) 495 μM ($n = 5$); (▲) 825 μM ($n = 4$). Each point represents the mean with vertical lines showing s.e.mean.

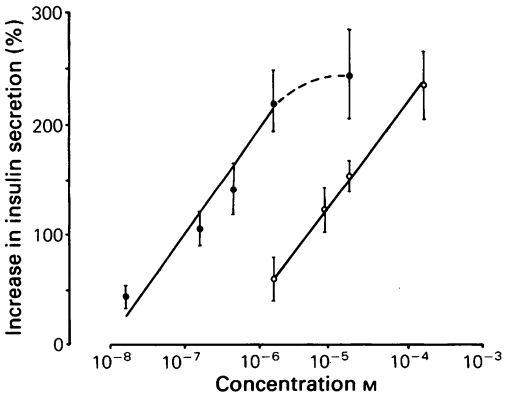

Figure 2 Concentration-response curves for 2-methylthio ATP- (●) and ATP- (○) induced insulin secretion. Each point represents the mean of 4 to 5 experiments and the vertical lines indicate the s.e.mean. Solid lines represent the calculated regression lines.

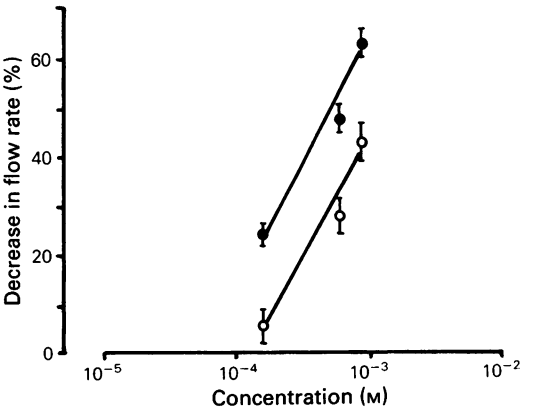

Figure 4 Concentration-response curves for the effects of 2-methylthio ATP (●) and ATP (○) on pancreatic flow rate. Each point represents the mean of the maximal fall in flow rate drop ($n = 5$); vertical lines show s.e.mean.

Discussion

This study shows that 2-methylthio ATP induces, as does ATP, a biphasic insulin release and also a transient decrease of flow rate from rat isolated perfused pancreas.

The question arises whether these effects are mediated through breakdown products of these nucleotides. Previous results from this laboratory have shown that the effect of ATP was not due to metabolic products such as adenosine and acted via P_2-purinoceptors. In fact, ATP was 100 times more potent than adenosine in inducing insulin secretion (Loubatières-Mariani *et al.*, 1979) and stable ATP analogues, such as α,β-methylene ATP and α,β-methylene ADP, were as potent as ATP (Chapal & Loubatières-Mariani, 1981a). Besides, the insulin secretory effect of ATP was not antagonized by theophylline, a P_1-receptor antagonist; in contrast, it was counteracted by 2-2'-pyridylisatogene (PIT), a specific P_2 antagonist on the isolated pancreas preparation of the rat (Chapal & Loubatières-Mariani, 1981b). Thus, ATP did not act through adenosine receptors but through P_2-purinoceptors; the same probably holds true for 2-methylthio ATP. With respect to the effect of these nucleotides on the flow rate of isolated pancreas, only the triphosphate derivatives decreased the flow rate by vasoconstriction; adenosine increased the flow rate by vasodilatation (Chapal & Loubatières-Mariani, 1983; Soulaymani *et al.*, 1985). Thus, ATP and probably 2-methylthio ATP act *per se* on pancreatic flow rate through P_2-purinoceptors and not after conversion to adenosine.

It may be noted that the insulin secretory and vasoconstrictor effects of 2-methylthio ATP were observed at totally different concentrations. The insulin secretory effect was obtained at nanomolar concentrations (16.5–1650 nM) whereas the vasoconstrictor effect occurred only at micromolar concentrations (165–825 μM). 2-Methylthio ATP exhibits a much higher selectivity for the P_2-receptors of the β cell than for those of the pancreatic vascular bed.

Furthermore, the present results show a difference in relative potency between ATP and its 2-substituted analogue in inducing those two effects and suggest that two P_2-purinoceptors are implicated. Indeed, as for the insulin secretory effect, 2-methylthio ATP at concentrations ranging from 16.5 to 1650 nM induced a concentration-dependent response, and was 45 times more effective than ATP. In contrast, on the pancreatic flow rate, 2-methylthio ATP and ATP are effective in the same range of micromolar concentrations, 2-methylthio ATP being only twice as potent as ATP.

Such differences in relative potency of ATP and its 2-substituted analogue have been reported in other tissues. 2-Methylthio ATP was shown to be 50 to 200 fold more potent than ATP at relaxing the guinea-pig isolated taenia coli (Gough *et al.*, 1973; Satchell & Maguire, 1975; Burnstock *et al.*, 1983), the longitudinal muscle of the rabbit portal vein (Kennedy & Burnstock, 1985) and the pig aorta (Martin *et al.*, 1985). However, ATP and 2-methylthio ATP have been shown to have about the same potency in increasing the force of contraction of the frog ventricle, the contraction of guinea-pig urinary bladder and vas deferens (Burnstock *et al.*, 1983; 1985). It is obvious that the P_2-purinoceptors do not form a homogeneous group. Thus, Burnstock & Kennedy (1985), suggested that P_2-purinoceptors may be separated into two subtypes on the basis of the rank order of agonist potency of structural analogues of ATP. According to this classification, a P_{2Y} receptor mediating an increase of insulin secretion would be present on β cells of the rat pancreas, since we observed that 2-methylthio ATP was much more potent than ATP. In contrast a P_{2X} receptor mediating a decrease of flow rate of pancreatic vascular bed would be present on the vessels since we observed that ATP was almost as potent as 2-methylthio ATP.

As stated in the introduction, the agonist potency order of ATP and its phosphate-modified analogues previously established gave additional evidence for the presence of P_{2Y} and P_{2X} purinoceptors in rat pancreas.

In conclusion, the present experiments provide evidence for a P_{2Y}-purinoceptor on the insulin secreting cell and a P_{2X} purinoceptor on the vascular bed of the rat pancreas.

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