Br. J. Pharmacol. (1991), **102**, 627–630 © Macmillan Press Ltd, 1991

Adenosine-5′-O-(2-thiodiphosphate) is a potent agonist at P_2 purinoceptors mediating insulin secretion from perfused rat pancreas

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

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

1 The effects of a P_2 purinoceptor agonist, adenosine 5′-O-(2-thiodiphosphate) (ADP-β-S) have been studied on insulin secretion and flow rate of the isolated perfused pancreas of the rat.

2 In the presence of a moderately stimulating glucose concentration (8.3 mM), ADP-β-S (4.95–495 nM) evoked a biphasic insulin response in a concentration-dependent manner. A comparison of relative potency between ADP-β-S and adenosine 5′-triphosphate (ATP) showed that ADP-β-S was 100 times more potent than ATP. On the other hand, in the presence of a non stimulatory glucose concentration (4.2 mM), ADP-β-S (165 nM) did not modify the basal insulin secretion.

3 ADP-β-S, at concentrations effective on insulin secretion and also at higher concentrations (1.65 and 16.5 μM), provoked an increase of the pancreatic flow rate in a concentration-dependent manner.

4 Our results show that ADP-β-S is a potent insulin secretory P_2 purinoceptor agonist. As it is resistant to hydrolysis it might be useful in studying the effect of activation of the P_2 purinoceptor of β cells on insulin secretion *in vivo*.

Introduction

Adenine nucleotides can influence numerous biological processes by acting on specific receptors on the cellular surface (Burnstock & Brown, 1981; Gordon, 1986). Previously we have shown that exogenous adenosine triphosphate and diphosphate (ATP and ADP) stimulated insulin release from rat pancreatic β cells, 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} largely on the basis of the rank order of agonist potency of structural analogues of ATP (Burnstock & Kennedy, 1985). The agonist potency order at P_{2x} purinoceptors is α,β-methylene ATP > ATP ≈ 2-methylthio ATP whereas at P_{2y} purinoceptors the order is 2-methylthio ATP ≫ ATP ≥ α,β-methylene ATP. According to this classification we have characterized the P_2 purinoceptor present on rat β cells as a P_{2y} subtype, 2-methylthio ATP being more potent than ATP in stimulating insulin secretion (Bertrand *et al.*, 1987).

ADP analogues with modification on the terminal phosphate have been reported to be more effective (Burnstock *et al.*, 1984) or selective (Hourani *et al.*, 1988) P_{2y} receptor agonists. Furthermore, in contrast to 2-methylthio ATP, these ADP analogues are more resistant to ectonucleotidases than natural agonists (Welford *et al.*, 1986).

In the present study, we have investigated the effects of adenosine-5′-O-(2-thiodiphosphate) (ADP-β-S), a phosphorothioate analogue of ADP, on insulin secretion from the isolated perfused pancreas of the rat. The insulin secretory effect of ADP-β-S was compared with that of ATP, taken as reference in previous experiments. The present work shows that ADP-β-S is a very potent agonist at P_{2y} purinoceptors mediating insulin release.

Methods

Our experiments were performed on male Wistar rats fed *ad libitum* and weighing 330 to 350 g. The surgical procedure for the rat isolated perfused pancreas has been described previously (Loubatières *et al.*, 1969; Bertrand *et al.*, 1986). The pancreas was perfused at 37.5°C with a Krebs Ringer bicarbonate buffer containing pure bovine serum albumin (fraction V) 2 g l^{-1} and glucose 8.3 or 4.2 mM; the pH was about 7.4. Each organ was perfused at a constant pressure (Bertrand *et al.*, 1987).

ADP-β-S or ATP, was infused for 30 min from time 45 min. Samples were taken every min during the first 6 min, then at 8, 10, 15, 20 and 30 min. Insulin in the pancreatic effluent was assayed by the method of Herbert *et al.* (1965) using the antibody supplied by Miles Laboratories (Paris). [^{125}I]-insulin was obtained from International CIS (Gif-Sur-Yvette, France). Pure rat insulin (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 insulin secretion and flow rate, we determined respectively the increase of mean insulin output and flow rates over the 30 min of nucleotide infusion as follows: (nucleotide AUC − control AUC)/30 (AUC = area under the curve). The values obtained were plotted as a function of the logarithm of ADP-β-S or ATP concentrations.

For statistical studies of changes in pancreatic flow rate, the AUC were submitted to analysis of variance followed by the multiple comparison test (Zar, 1974).

Drugs

Adenosine-5′-triphosphate (ATP) and adenosine-5′-O-(2-thiodiphosphate) (ADP-β-S) were obtained from Boehringer Mannheim. ATP was the sodium salt and ADP-β-S was the lithium salt.

Results

Effect of adenosine-5′-O-(2-thiodiphosphate) on insulin secretion

In the presence of 8.3 mM glucose (controls), insulin release increased slightly with time (Figure 1).

[1] Author for correspondence.

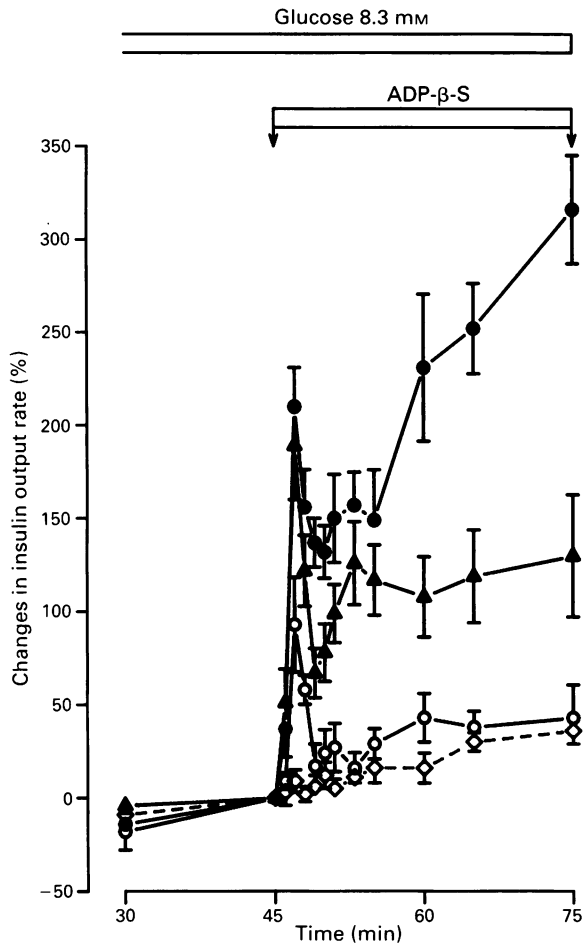

Figure 1 Effects of increasing concentrations of adenosine-5'-O-(2-thiodiphosphate) (ADP-β-S) on insulin secretion from the isolated perfused pancreas of the rat: (○) 49.5 nM (n = 4); (▲) 165 nM (n = 7); (●) 495 nM (n = 7), controls (◇) (n = 6). The insulin output rate (ng min^{-1}) at 45 min for each set of experiments was: 35.25 ± 4.32; 30.20 ± 5.45; 31.47 ± 4.20; 32.91 ± 8.09 respectively. Each point represents the mean with s.e.mean shown by vertical lines.

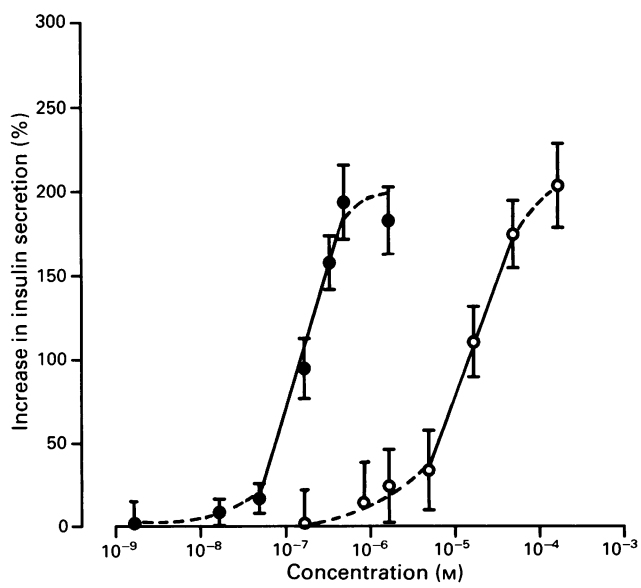

Figure 2 Concentration-response curves for adenosine-5'-O-(2-thiodiphosphate) (ADP-β-S, ●)- and adenosine 5'-triphosphate (ATP, ○)-induced insulin secretion. Each point represents the mean of 4–7 experiments and vertical lines indicate the s.e.mean. Solid lines represent the calculated regression lines.

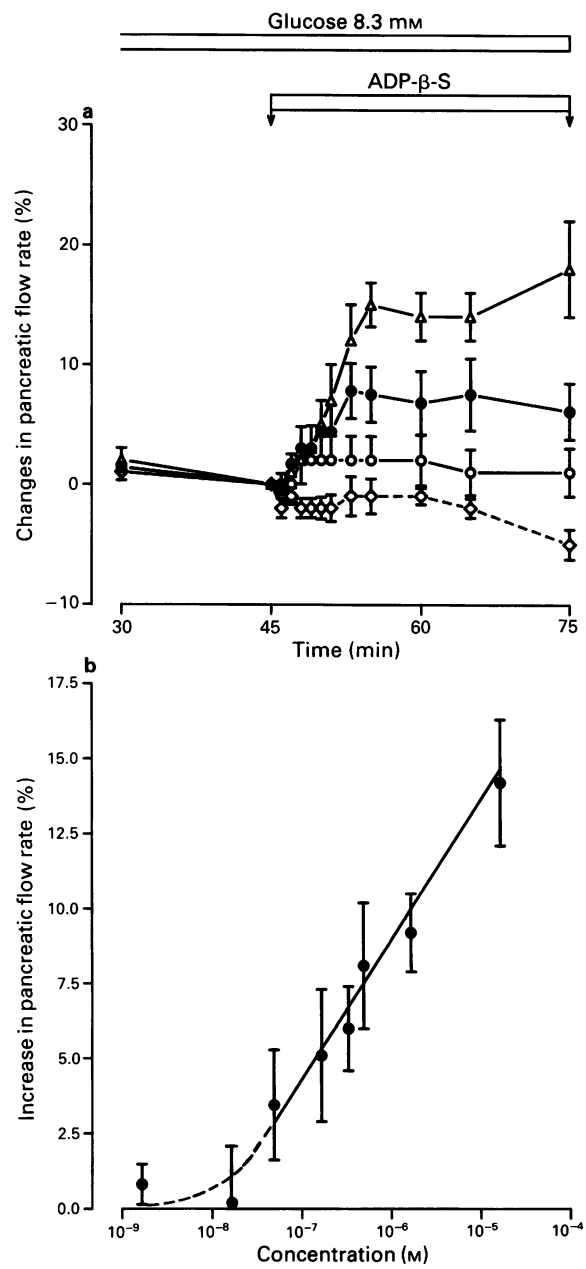

Figure 3 Effects of adenosine-5'-O-(2-thiodiphosphate) (ADP-β-S) on pancreatic flow rate of the rat: (a) (○) 49.5 nM (n = 4); (●) 495 nM (n = 7); (△) 16.5 μM (n = 6); controls (◇) (n = 6). (b) Concentration-response curve. Each point represents the mean with vertical lines showing s.e.mean.

The effects of ADP-β-S were studied at concentrations ranging from 1.65×10^{-9} to 1.65×10^{-5} M. At 1.65 and 16.5 nM ADP-β-S did not significantly affect insulin release. In the range 49.5–495 nM, ADP-β-S evoked a biphasic insulin response which was concentration-dependent (Figure 1). The kinetics of the insulin response were qualitatively similar to those of the response elicited by ATP (Loubatières-Mariani *et al.*, 1979). When it was used at 1.65 or 16.5 μM, insulin responses were not significantly different from those observed with 495 nM.

The concentration-response curves for ADP-β-S and ATP are shown in Figure 2. For each compound we carried out a linear regression analysis from the values of increase of mean insulin output rate. A comparison of potency was performed by the parallel line assay method (Armitage, 1980). The two concentration-response curves did not deviate significantly from parallelism; ADP-β-S was 100 fold more potent than ATP with [65–150] for 95% confidence limits.

In the presence of a non stimulatory concentration of glucose (4.2 mM), ADP-β-S at 165 nM, (the concentration inducing the half-maximum effect in the presence of glucose 8.3 mM) did not influence basal insulin output rate. The insulin output during the 30 min of ADP-β-S infusion was 10.0 ± 1.9 ng versus 9.8 ± 1.2 ng in the controls.

Effect of adenosine-5'-O-(2-thiodiphosphate) on pancreatic flow rate

In the absence of ADP-β-S the pancreatic flow rate decreased slightly with time (Figure 3a).

ADP-β-S at 1.65 and 16.5 nM did not significantly modify the flow rate. At concentrations effective on insulin secretion (49.5–495 nM), it induced a progressive and slight increase of the pancreatic flow rate (Figure 3a) which reached a maximum 8–10 min after the start of the infusion and remained sustained. ADP-β-S at the highest concentration tested (16.5 μM) induced a vasodilator effect which was more pronounced. With ADP-β-S 49.5 nM, 165 nM, 495 nM and 16.5 μM the AUC were, respectively, 3044 ± 54 (NS), 3118 ± 71 ($P < 0.05$), 3183 ± 63 ($P < 0.01$), and 3364 ± 63 ($P < 0.001$) versus controls (2945 ± 17). The effect of ADP-β-S on the mean flow rate was concentration-dependent at the tested concentrations in the range 49.5 nM–16.5 μM (Figure 3b).

The effects of ATP on flow rate were studied at concentrations ranging from 0.165 to 165 μM. With ATP 0.165 μM the flow rate was not modified. At the higher concentrations (1.65–49.5 μM) the flow rate responded differently according to the pancreas; it was either not changed or slightly increased. At 1.65, 4.95 and 49.5 μM the AUC were respectively 3089 ± 85 (NS), 3110 ± 102 (NS) and 3215 ± 107 (NS) versus 2940 ± 16 in controls. Only the concentration of 16.5 μM induced a significant increase of the flow rate: the area under the curve was 3241 ± 110 ($P < 0.05$) versus controls. This effect was not observed at the highest concentration tested (165 μM): the AUC was 2941 ± 84.

Discussion

This study shows that ADP-β-S, like ATP, induces a biphasic insulin release when rat isolated pancreas is perfused in the presence of a moderately stimulatory concentration of glucose (8.3 mM). On the other hand, ADP-β-S itself is ineffective on basal insulin secretion in the presence of a non stimulatory glucose concentration (4.2 mM). Thus, this agent is a potentiator of glucose-induced insulin release. Previous results have demonstrated that ATP or ADP, which were ineffective with glucose at a concentration of 4.2 mM (Bertrand *et al.*, 1989) stimulated insulin release in the presence of glucose 8.3 mM, via P_2 purinoceptors (Loubatières-Mariani *et al.*, 1979); the P_2 purinoceptor involved was characterized as a P_{2y} subtype, since 2-methylthio ATP was more potent than ATP (Bertrand *et al.*, 1987). The present work shows that an ADP analogue, ADP-β-S, is a potent insulin secretory agent in the presence of a stimulatory glucose concentration. ADP-β-S, at concentrations ranging from 4.95×10^{-8} M to 4.95×10^{-7} M induced a concentration-dependent response and was 100 times more potent than ATP. Like 2-methylthio ATP, ADP-β-S exhibits a much higher potency than ATP at P_{2y} purinoceptors of the β cell. Whereas 2-methylthio ATP is degraded at the same rate as ATP, ADP-β-S has been reported to be resistant to degradation (Welford *et al.*, 1986). However, the high potency of ADP-β-S on β cells appears not to be related to its stability, since another stable analogue of ADP, α,β-methylene ADP, has been shown to display nearly the same activity as ATP on insulin secretion (Chapal & Loubatières-Mariani, 1981). Furthermore no correlation has been reported between the rate of breakdown of an agonist and its pharmacological potency at P_{2y} receptors (Welford *et al.*, 1986). ADP-β-S has been shown to be more potent than ATP or ADP at P_{2y} purinoceptors involved in relaxation of the guinea-pig isolated taenia coli (Burnstock *et al.*, 1984) and in activation of the turkey erythrocyte phospholipase C (Boyer *et al.*, 1989). On the other hand, ADP-β-S was equipotent with ATP or ADP at P_{2x} purinoceptors mediating contraction of the guinea-pig urinary bladder and vas deferens (Burnstock *et al.*, 1984; Fedan *et al.*, 1986). Since ADP-β-S appears to be a more potent agonist at the P_{2y} purinoceptor, our results provide further pharmacological evidence for a P_{2y} purinoceptor on rat β cells.

We have previously shown that the pancreatic vascular bed was transiently constricted by ATP or ADP at very high concentrations (> 165 μM) (Chapal & Loubatières-Mariani, 1983). The agonist potency order of ATP and its structural analogues on the decrease of flow rate: α,β-methylene ATP > β,γ-methylene ATP > 2-methylthio ATP > ATP indicated the involvement of a P_{2x} purinoceptor (Chapal & Loubatières-Mariani, 1983; Bertrand *et al.*, 1987). In our study, ADP-β-S, even at the highest concentrations, elicited in contrast, an increase in pancreatic flow rate which was concentration-dependent from 4.95×10^{-8} M. Nevertheless, it should be noted that ADP-β-S at the concentration (495 nM) inducing a maximum effect on insulin secretion, did not induce a maximum vasodilator effect, since a much more pronounced vasodilatation was observed at the highest concentration tested (16.5 μM). A slight but significant vasodilator effect occurred with ATP only at 16.5 μM but could not be observed at the high concentration of 165 μM. The disappearance of the vasodilator effect could be explained by the opposing vasoconstrictor effect of ATP, via P_{2x} purinoceptors, which becomes apparent at high concentrations, as previously shown (Chapal & Loubatières-Mariani, 1983); such a mechanism has also been proposed in the mesenteric bed of the rat in which tone has been increased by noradrenaline (Ravelic & Burnstock, 1988). In fact, ATP can mediate vessel relaxation or constriction through endothelium P_{2y} purinoceptors and smooth muscle P_{2x} purinoceptors respectively (Burnstock, 1987; Pearson & Gordon, 1989) but it could also act indirectly, after hydrolysis into adenosine, on P_1 purinoceptors mediating vasodilatation. Concerning the pancreatic vascular bed, adenosine has been reported to increase the flow rate by acting on P_1 purinoceptors (Soulaymani *et al.*, 1985). However, it seems unlikely that vasodilatation elicited by ADP-β-S involves an action via adenosine, since this ADP analogue is resistant to hydrolysis. So ADP-β-S, a stable and more specific P_{2y} agonist evoked, at the concentrations used, only a vasodilator effect, whereas ATP exhibited more complex effects.

We have previously suggested that ATP and ADP, via P_2 purinoceptor activation, could exert an important role in the control of the β cell by potentiating acetylcholine (Bertrand *et al.*, 1986) and glucose (Bertrand *et al.*, 1989) stimulations. Furthermore we have shown that 2-methylthio ATP infused directly into the dog pancreatico-duodenal artery (this procedure was used because of the rapid metabolism of this analogue) induced an insulin secretion sufficient to provoke a reduction of glycaemia (Ribes *et al.*, 1988). Since ADP-β-S is a more stable and potent insulin secretory P_{2y} purinoceptor agonist, it might therefore be a useful agent to stimulate insulin secretion and decrease glycaemia *in vivo*.

The excellent technical assistance of M. Roye, R. Assié and M.F. Courty is gratefully acknowledged. This work was supported by Grant No. 882008 from INSERM.

References

ARMITAGE, P. (1980). Biological assay. In *Statistical Methods in Medical Research*. ed. Armitage, P. pp. 442–456. Oxford: Blackwell.

BERTRAND, G., CHAPAL, J. & LOUBATIERES-MARIANI, M.M. (1986). Potentiating synergism between adenosine diphosphate or triphosphate and acetylcholine on insulin secretion. *Am. J. Physiol.*, **251**, E416–E421.

BERTRAND, G., CHAPAL, J., LOUBATIERES-MARIANI, M.M. & ROYE, M. (1987). Evidence for two different P_2-purinoceptors on β cell and pancreatic vascular bed. *Br. J. Pharmacol.*, **91**, 783–787.

BERTRAND, G., GROSS, R., CHAPAL, J. & LOUBATIERES-MARIANI, M.M. (1989). Difference in the potentiating effect of adenosine triphosphate and α,β-methylene ATP on the biphasic insulin response to glucose. *Br. J. Pharmacol.*, **98**, 998–1004.

BOYER, J.L., DOWNES, C.P. & HARDEN, T.K. (1989). Kinetics of activation of phospholipase C by P_{2y} purinergic receptor agonists and guanine nucleotides. *J. Biol. Chem.*, **264**, 884–890.

BURNSTOCK, G. (1987). Local control of blood pressure by purines. *Blood Vessels*, **24**, 156–160.

BURNSTOCK, G. & BROWN, C.M. (1981). An introduction to purinergic receptors. In *Purinergic Receptors*. ed. Burnstock, G.W. pp. 1–45. London: Chapman and Hall.

BURNSTOCK, G., CUSAK, N.J. & MELDRUM, L.A. (1984). Effects of phosphorothioate analogues of ATP, ADP and AMP on guinea-pig taenia coli and urinary bladder. *Br. J. Pharmacol.*, **82**, 369–374.

BURNSTOCK, G. & KENNEDY, C. (1985). Is there a basis for distinguishing two types of P_2-purinoceptor? *Gen. Pharmacol.*, **16**, 433–440.

CHAPAL, J. & LOUBATIERES-MARIANI, M.M. (1981). Effects of phosphate-modified adenine nucleotide analogues on insulin secretion from perfused rat pancreas. *Br. J. Pharmacol.*, **73**, 105–110.

CHAPAL, J. & LOUBATIERES-MARIANI, M.M. (1983). Evidence for purinergic receptors on vascular smooth muscle in the pancreas. *Eur. J. Pharmacol.*, **87**, 423–430.

FEDAN, J.S., HOGABOOM, G.K. & O'DONNELL, J.P. (1986). Further comparison of contractions of the smooth muscle of the guinea-pig isolated vas deferens induced by ATP and related analogs. *Eur. J. Pharmacol.*, **129**, 279–291.

GORDON, J.L. (1986). Extracellular ATP: effects, sources and fate. *Biochem. J.*, **233**, 309–319.

HERBERT, V., LAW, K.S., GOTTLIEB, C.W. & BLEICHER, S.J. (1965). Coated charcoal immunoassay of insulin. *J. Clin. Endocr.*, **25**, 1375–1384.

HOURANI, S.M.O., WELFORD, L.A., LOIZOU, G.D. & CUSAK, N.J. (1988). Adenosine 5'-(2-fluorodiphosphate) is a selective agonist at P_2-purinoceptors mediating relaxation of smooth muscle. *Eur. J. Pharmacol.*, **147**, 131–136.

LOUBATIERES, A.L., MARIANI, M.M., DE MALBOSC, H., RIBES, G. & CHAPAL, J. (1969). Etude expérimentale d'un nouveau sulfamide hypoglycémiant particulièrement actif, le HB 419 ou glibenclamide. I. Action bêtacytotrope et insulino-sécrétrice. *Diabetologia*, **5**, 1–10.

LOUBATIERES-MARIANI, M.M., CHAPAL, J., LIGNON, F. & VALETTE, G. (1979). Structural specificity of nucleotides for insulin secretory action from the isolated perfused rat pancreas. *Eur. J. Pharmacol.*, **59**, 277–286.

PEARSON, J.D. & GORDON, J.L. (1989). P_2 purinoceptors in the blood vessel wall. *Biochem. Pharmacol.*, **38**, 4157–4163.

RALEVIC, V. & BURNSTOCK, G. (1988). Actions mediated by P_2-purinoceptorsubtypes in the isolated perfused mesenteric bed of the rat. *Br. J. Pharmacol.*, **95**, 637–645.

RIBES, G., BERTRAND, G., PETIT, P. & LOUBATIERES-MARIANI, M.M. (1988). Effects of 2-methylthio ATP on insulin secretion in the dog *in vivo*. *Eur. J. Pharmacol.*, **155**, 171–174.

SOULAYMANI, R., GROSS, R., CHAPAL, J. & LOUBATIERES-MARIANI, M.M. (1985). Effet dilatateur de l'adénosine sur les vaisseaux du pancréas de rat. *C.R. Soc. Biol.*, **179**, 371–374.

WELFORD, L.A., CUSAK, L.N. & HOURANI, S.M.O. (1986). ATP analogues and the guinea-pig taenia coli: a comparison of the structure-activity relationships of ectonucleotidases with those of the P_2-purinoceptor. *Eur. J. Pharmacol.*, **129**, 217–224.

ZAR, J.H. (1974). *Biostatistical Analysis*. Englewood Cliffs, N.J.: Prentice-Hall.

(Received June 11, 1990
Revised October 30, 1990
Accepted November 5, 1990)