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Stimulation of insulin secretion and improvement of glucose tolerance in rat and dog by the P_{2y}-purinoceptor agonist, adenosine-5′-O-(2-thiodiphosphate)

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1 *In vivo* effect of a P_{2y}-purinoceptor agonist, adenosine-5′-O-(2-thiodiphosphate) (ADPβS), on insulin secretion and glycaemia were studied both in rats and dogs.

2 In anaesthetized rats, i.v. administered ADPβS (0.2 mg kg^{-1}) produced an insulin response dependent on the nutritional state of the animals, since we observed only a transient increase in overnight-fasted rats and a sustained insulin secretion followed by a reduction in plasma glucose levels in fed rats. During an i.v. glucose tolerance test, ADPβS enhanced insulin release and thus increased the glucose disappearance rate.

3 In anaesthetized fasted dogs, i.v. administered ADPβS (0.1 mg kg^{-1}) increased pancreaticoduodenal insulin output and slightly decreased blood glucose levels.

4 In conscious fasted dogs, orally administered ADPβS (0.1 mg kg^{-1}) transiently increased insulinemia and punctually reduced glycaemia. Furthermore, during an oral glucose tolerance test, orally administered ADPβS at the same dose markedly enhanced insulin secretion and consequently reduced the hyperglycaemia.

5 In conclusion, the P_{2y}-agonist, ADPβS, is a potent insulin secretagogue *in vivo*, improves glucose tolerance and is effective after oral administration. Thus, the P_{2y}-purinoceptors of the β cell may be a target for new antidiabetic drugs.

Keywords: P_{2y}-purinoceptors; adenosine-5′-O-(2-thiodiphosphate) (ADPβS); insulin secretion; glucose tolerance in rat, in dog

Introduction

The insulin stimulating effect of adenine nucleotides (ATP, ADP) and structural analogues has been previously well established on rat isolated perfused pancreas (Loubatières-Mariani *et al.*, 1979; Chapal & Loubatières-Mariani, 1981). These agents act on purinoceptors which have been characterized as P_2, according to Burnstock's classification (1978). By use of 2-methylthio ATP, these receptors have been shown to belong to the P_{2y} subtype (Bertrand *et al.*, 1987). Recently a specific and more stable P_{2y}-agonist, adenosine-5′-O-(2-thiodiphosphate) (ADPβS), was found to be one hundred times more potent than ATP, and effective in the nanomolar range in stimulating insulin secretion *in vitro* on perfused rat pancreas (Bertrand *et al.*, 1991).

The expected consequence of an insulin stimulation *in vivo* is a decrease in glycaemia. Such an effect has been previously observed in dogs with a P_{2y}-agonist, 2-methylthio ATP; however, because of the rapid metabolism of this drug, it was necessary to infuse it *in situ*, directly into the pancreaticoduodenal artery of the dog (Ribes *et al.*, 1988). The potential interest of P_{2y}-agonists in diabetes therapy is linked to their insulin-stimulating effect; this interest would be greatly enhanced by their ability to exert this effect after peripheral intravenous and, more interestingly, after oral administration. So, in this work we investigated the *in vivo* effects of the more stable P_{2y}-agonist, ADPβS, on insulin secretion and glycaemia in rats and dogs.

Methods

General experimental conditions

All the experiments were performed *in vivo*. Male Wistar rats weighing about 380–450 g and male mongrel dogs weighing 12–22 kg were used.

In vivo *experiments in anaesthetized animals*

Rats were anaesthetized with an i.p. injection of pentobarbitone (60 mg kg^{-1}) and maintained on a heated operating surface. A catheter, filled with heparin saline to prevent blood clotting, was inserted into each jugular vein. The test substances were injected through one catheter and blood samples were taken from the other one. In all the experiments, after a 30 min resting period, 3 samples (0.5 ml over 1 min) were taken to determine basal plasma insulin and glucose levels. In all cases the solution of ADPβS was injected slowly at time 0 over 1.30 min; the drug was diluted in 0.5 ml saline.

The effects of ADPβS (0.2 mg kg^{-1}) were tested both in overnight fasted and fed rats. Furthermore the effect of ADPβS on an intravenous glucose tolerance test (IVGTT) was performed in fed rats. For this purpose a mixture of glucose (0.5 g kg^{-1}) plus ADPβS (0.2 mg kg^{-1}) diluted in 0.5 ml saline was injected for 1.30 min. For each set of experiments, control experiments were performed with i.v. saline or glucose alone.

Adult dogs fasted for 16 h were anaesthetized with pentobarbitone (30 mg kg^{-1}, i.v.). The animals were given heparin intravenously (5 mg kg^{-1}) and provided with a venous pancreaticoduodenal by-pass. For this purpose, after a median laparotomy a T-shaped catheter was inserted into the superior pancreaticoduodenal vein just at its exit from the pancreas (Hillaire-Buys *et al.*, 1984). Blood was sampled from the pancreaticoduodenal vein in order to measure insulin concentration. For this, the clamp was taken off the perpendicular part of the venous T-shaped catheter, and the blood stream towards the portal vein was clamped; blood (5 ml) for pancreatic hormone evaluation was collected in a graduated tube and the time of sampling was measured. Thus, it was possible to determine the venous pancreaticoduodenal blood flow. Thereafter the blood stream towards the portal vein was re-established. Insulin output rate was determined by multiplying the concentration of the hormone

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in the plasma by the venous blood flow corrected by the haematocrit. Blood glucose concentration was continuously recorded from a peripheral vein with a Technicon autoanalyzer. Femoral arterial blood pressure was recorded with a Ludwig's manometer. After a 60 min equilibration period, ADPβS at a dose of 0.1 mg kg^{-1} was injected for 1 min into a saphenous vein.

In vivo *experiments in conscious dogs*

Two groups of experiments were performed in dogs fasted for 16 h: (1) ADPβS was administered by intragastric intubation at a dose of 0.1 mg kg^{-1} diluted in 10 ml saline; (2) oral glucose tolerance tests (OGTT) were performed. A mixture of glucose (1 g kg^{-1}) plus ADPβS (0.1 mg kg^{-1}) (in 100 ml saline) was administered for 1 min by intragastric intubation. In these experiments, blood samples were taken from a jugular vein in order to evaluate blood glucose and plasma insulin levels.

Assays

In rats, plasma glucose levels were immediately determined by an enzymatic method (Trinder, 1969). In dogs, blood glucose was measured with a Technicon autoanalyzer (Alric *et al.*, 1965).

Insulin concentrations were measured by the method of Herbert *et al.* (1965) using an antibody supplied by Miles Laboratories (Paris). [^{125}I]-insulin was obtained from International CIS (Gif-sur-Yvette, France); the standard used was rat insulin (Novo, Copenhagen, Denmark) the biological activity of which was 22.3 μu ng^{-1}. The intra- and inter-assay coefficients of variation were respectively 9% and 13.5%. The analytical sensitivity was 2.2 μu ml^{-1}.

Data analysis

Data are expressed as mean values ± s.e.mean. All results were submitted to analysis of variance followed by the multiple comparison test (Zar, 1974). For glucose tolerance tests the areas under the curves (AUC) were calculated.

Drug

Adenosine-5'-O-(2-thiodiphosphate) (ADPβS) was purchased from Boehringer Mannheim. It was the lithium salt.

Results

In vivo *effects of i.v. administered ADPβS to anaesthetized rats*

In overnight fasted rats, i.v. administration of ADPβS at the dose of 0.2 mg kg^{-1} induced an immediate but transient increase in insulin secretion (Figure 1). No significant effect on plasma glucose levels was recorded.

In fed rats, i.v. administration of ADPβS at the same dose (0.2 mg kg^{-1}) (Figure 2) evoked a biphasic insulin response: plasma insulin levels rose from 67 ± 10 to 158 ± 16 μu ml^{-1} at 3 min ($P<0.001$); after the first phase in a peak form, the insulin levels remained elevated throughout the 90 min experiment. This sustained insulin response induced a progressive decrease in plasma glucose levels statistically significant from 30 min ($P<0.01$). It should be noted that basal plasma glucose and insulin levels were significantly increased ($P<0.01$) as compared with those of overnight fasted rats (7.90 ± 0.22 versus 7.10 ± 0.17 mM and 67 ± 10 vs 41 ± 9 μu ml^{-1}) at − 2 min.

In fed rats, we tested the effects of ADPβS on the IVGTT (Figure 3). After the i.v. glucose injection alone (0.5 g kg^{-1}), the plasma glucose concentration at 3 min was 17.50 ± 0.77 mM; it then progressively returned to basal

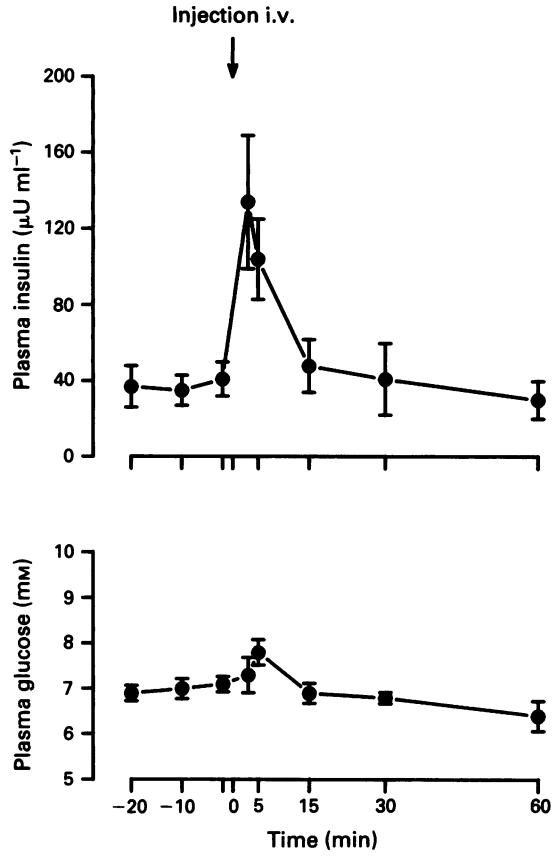

Figure 1 Effects of an intravenous injection of adenosine-5'-O-(2-thiodiphosphate) (ADPβS) 0.2 mg kg^{-1} on plasma insulin and glucose in anaesthetized rats that had been fasted overnight (●). Data are expressed as mean values ± s.e.mean as obtained from six different experiments.

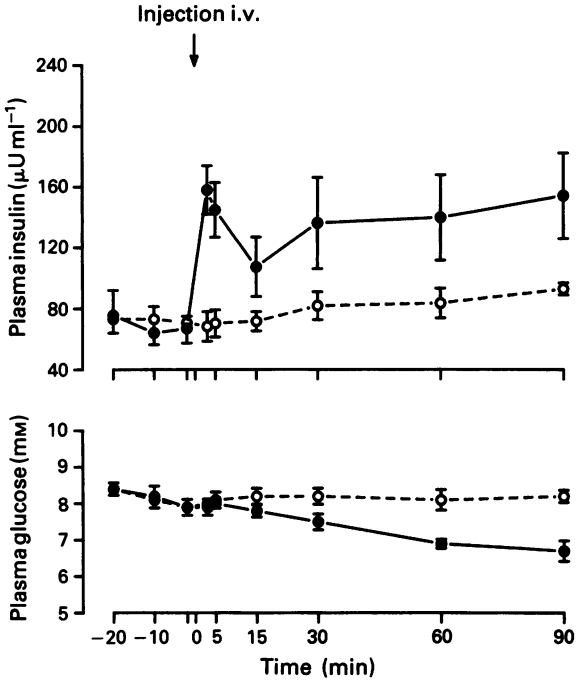

Figure 2 Effects of an intravenous injection of adenosine-5'-O-(2-thiodiphosphate) (ADPβS) 0.2 mg kg^{-1} on plasma insulin and glucose in anaesthetized fed rats (●). Control animals received i.v. saline (○). Data are expressed as mean values ± s.e.mean as obtained from six different experiments.

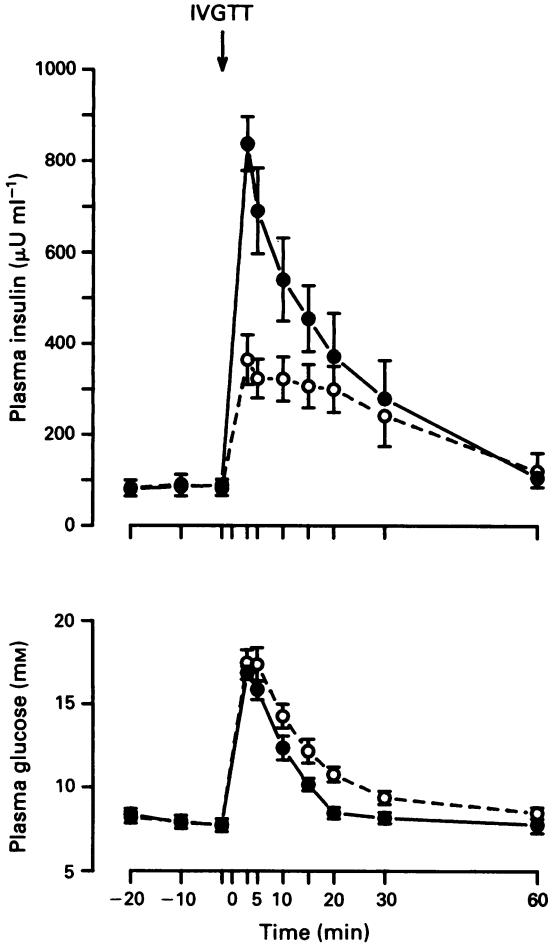

Figure 3 Effects of an intravenous injection of adenosine-5'-O-(2-thiodiphosphate) (ADPβS) (0.2 mg kg^{-1}) on the increases in plasma insulin and glucose levels in response to i.v. glucose injection (IVGTT) (0.5 g kg^{-1}) in fed rats (●). Control animals received i.v. glucose injection alone (○). Data are mean values ± s.e.mean as obtained from six different experiments.

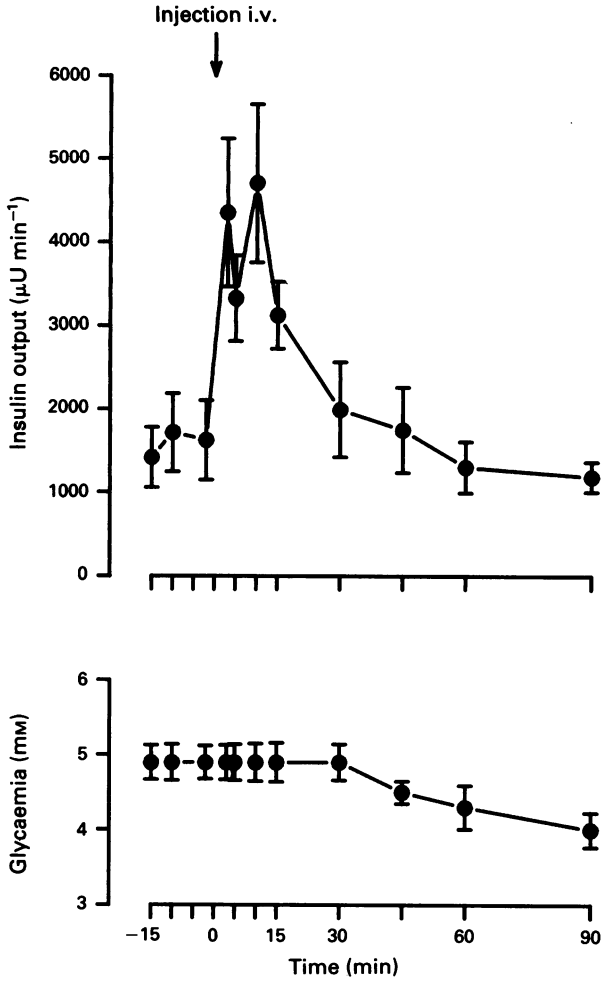

Figure 4 In anaesthetized fasted dogs, provided with a T shaped catheter in the pancreaticoduodenal vein, effects of an intravenous injection of adenosine-5'-O-(2-thiodiphosphate) (ADPβS) (0.1 mg kg^{-1}) on pancreatic insulin output and glycaemia. Data are expressed as mean values ± s.e.mean as obtained from five different experiments.

values which were reached at 60 min. The rise in glucose levels was associated with an increase in plasma insulin concentrations which rose to 366 ± 55 μu ml^{-1} at 3 min and remained significantly high up to 20 min. When ADPβS (0.2 mg kg^{-1}) was added, the increase in plasma insulin levels was significantly higher: 839 ± 59 μu ml^{-1} at min 3 ($P<0.001$ vs controls receiving glucose alone). The integrated insulin response (AUC) during the first 20 min was 8770 ± 1142 vs 4426 ± 656 μu ml^{-1} in controls ($P<0.01$). At 3 min the maximum hyperglycaemia was not significantly different from that obtained with glucose alone (16.90 ± 0.39 mM). However, the return to basal values was more rapid; these were already reached from 20 min. The integrated glucose response (AUC) during the first 20 min was 28% smaller than in controls ($P<0.01$). The glucose disappearance rate (K_G; slope of the logarithm of glucose levels between 3 and 20 min) was higher ($P<0.01$), in rats which received ADPβS (4.12 ± 0.02% min^{-1}) than in control rats (3.05 ± 0.23% min^{-1}).

In vivo *effects of i.v. administered ADPβS to anaesthetized fasted dogs*

Intravenous injection of ADPβS (0.1 mg kg^{-1}) induced an immediate and transient increase in pancreaticoduodenal insulin output (Figure 4). The increase was statistically significant from 3 min to 15 min. Then the insulin output returned towards the starting values. This increase in insulin secretion was followed by a progressive reduction in blood glucose level. In these anaesthetized dogs, glycaemia which was 4.90 ± 0.22 mM before ADPβS administration reached 4.00 ± 0.23 mM at 90 min ($P<0.01$). The injection of ADPβS produced a short lasting diminution in the arterial blood pressure (at 3 min, $P<0.001$) (Table 1); at 5 min the arterial blood pressure had almost returned to the starting values. ADPβS did not significantly influence the pancreaticoduodenal venous blood flow.

In vivo *effects of orally administered ADPβS to conscious dogs*

The administration of ADPβS (0.1 mg kg^{-1}) by intragastric intubation to fasted dogs induced an increase in plasma insulin levels (Figure 5). This hyperinsulinemia appeared 10 min after the intubation ($P<0.01$) and was also observed at 15 min; the plasma insulin then returned to the basal value which was reached at 45 min. At 15 min a reduction in glycaemia was also noted ($P<0.01$).

For the OGTT (1 g kg^{-1}) (Figure 6) the increment in glycaemia reached + 3.17 ± 0.39 mM within 30 min. This hyperglycaemia evoked an increase in insulinemia (+ 44 ± 7 μu ml^{-1} at 30 min). When ADPβS (0.1 mg kg^{-1}) was added to the glucose solution, increments in plasma insulin levels were significantly higher from 5 min (+ 90 ± 13 μu ml^{-1} at

Table 1 Effects of an intravenous injection of adenosine-5'-O-(2-thiodiphoshate) (ADPβS, 0.1 mg kg^{-1}) on arterial blood pressure (mmHg) and venous pancreaticoduodenal blood flow (ml min^{-1}) in anaesthetized, fasted dogs

Min	− 15	− 10	− 2	3	5	10	15	30	45	60	90	120	50	180
Arterial blood pressure (5)	129 ± 7	129 ± 7	129 ± 7	*88 ± 9	119 ± 9	118 ± 8	118 ± 8	123 ± 8	125 ± 8	123 ± 9	121 ± 12	116 ± 12	121 ± 13	120 ± 13
Blood flow in pancreatico-duodenal vein (5)	14.6 ± 1.8	15.1 ± 1.9	14.6 ± 1.5	13.6 ± 1.8	14.0 ± 1.6	13.7 ± 1.2	13.5 ± 1.4	13.6 ± 1.6	13.9 ± 1.7	14.3 ± 1.0	13.9 ± 1.2	13.8 ± 1.4	13.6 ± 1.3	12.9 ± 2.1

*Blood pressure reduced, compared to other time points, $P<0.001$. The number of animals is given in parentheses.

30 min, $P<0.01$). Thus the area under the curve (AUC) for hyperinsulinemia during the first 90 min was greater (5244 ± 1115 versus 2860 ± 563 μu ml^{-1} with glucose alone, $P<0.05$). The integrated glucose response during these first 90 min was significantly reduced ($P<0.05$) in ADPβS-treated dogs (93.3 ± 13.4 versus 183.4 ± 31.9 mM in controls).

Discussion

The main result of this study is that the P_{2y}-purinoceptor agonist, ADPβS, stimulates insulin secretion and improves glucose tolerance not only when administered intravenously but also orally.

In anaesthetized rats, ADPβS injected into a peripheral vein evoked an increase in plasma insulin levels. The insulin stimulating effect of ADPβS appears to be dependent on the nutritional state of the animals. Indeed in overnight fasted rats, ADPβS (0.2 mg kg^{-1}) elicited only a transient insulin response without modification of glycaemia. In contrast, in fed rats, in which plasma glucose levels were higher than in overnight fasted rats, the same dose of ADPβS evoked a sustained insulin response with a reduction of glycaemia. Furthermore, in fed rats, this effect seems to be dependent on the dose since a two fold lower dose of ADPβS (0.1 mg kg^{-1}) evoked a transient insulin response insufficient to induce a decrease of plasma glucose levels (data not shown). The lack of effect of ADPβS in fasted conditions could limit the risk of

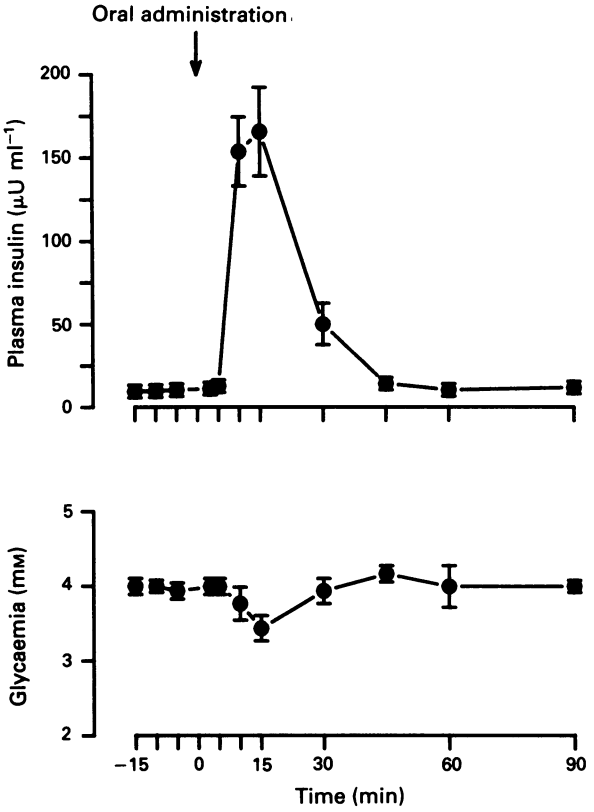

Figure 5 Effects of adenosine-5'-O-(2-thiodiphosphate) (ADPβS) at 0.1 mg kg^{-1} via intragastric intubation on plasma insulin and glycaemia in fasted conscious dogs. Data are expressed as mean values ± s.e.mean as obtained from five different experiments.

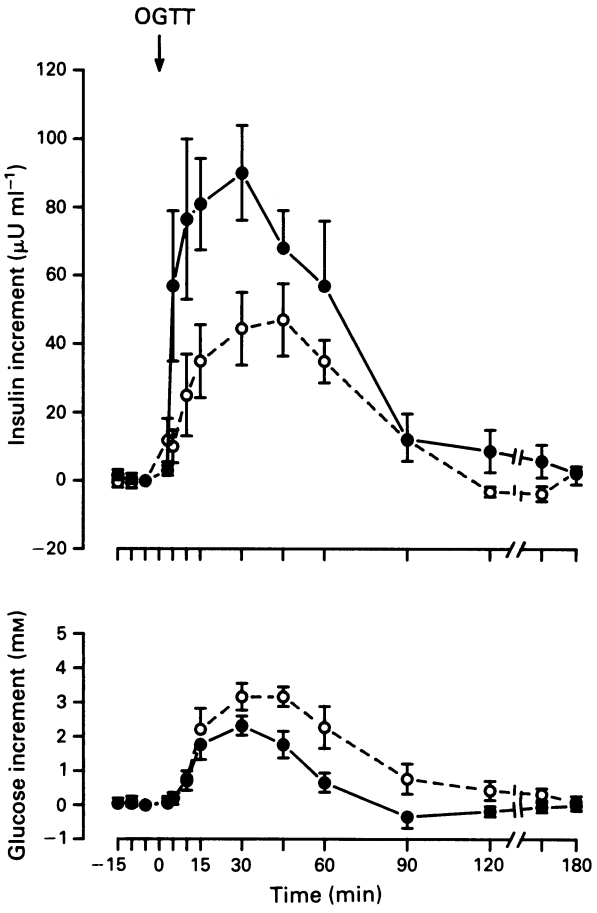

Figure 6 Increments in plasma insulin and glucose levels after oral glucose tolerance test (OGTT) in fasted conscious dogs, in response to glucose alone (1 g kg^{-1}) (○) or glucose (1 g kg^{-1}) + ADPβS (0.1 mg kg^{-1}) (●). Basal values of glycaemia and insulinemia (at min − 5) for dogs receiving glucose alone were 4.7 ± 0.2 mM and 10.1 ± 2.7 μu ml^{-1} respectively; they were 4.6 ± 0.1 mM and 9.7 ± 3.2 μu ml^{-1} respectively for dogs receiving glucose + ADPβS. Data are expressed as mean values ± s.e.mean as obtained from five different experiments.

hypoglycaemia. These *in vivo* findings are in agreement with previous *in vitro* experiments showing that the insulin stimulating effect of ADPβS was related to the glucose concentration (Bertrand *et al.*, 1991).

In anaesthetized fasted dogs, the peripheral intravenous injection of ADPβS induced an immediate but only transient increase in pancreaticoduodenal insulin which was followed by a slight reduction in blood glucose levels; these reached values comparable to basal glycaemia recorded in fasted conscious dogs. We have previously shown that another P_{2y}-agonist, 2-methylthio ATP, was able to induce insulin secretion and to reduce glycaemia slightly (Ribes *et al.*, 1988); however, because of its rapid degradation, this substance must be directly infused in the pancreaticoduodenal artery and thus is devoid of potential therapeutic interest. In contrast, ADPβS is a P_{2y}-agonist known to be resistant to ectonucleotidases (Welford *et al.*, 1986) and our results confirm the stability of this agent. It is known that purine nucleotides are metabolized in gut and largely converted to uric acid during passage across the intestinal mucosa (Stone & Simmonds, 1991) and therefore cannot exert systemic effects. In contrast, in the present study, the structural analogue, ADPβS, was effective after oral administration to conscious dogs, and, to our knowledge, it is the first time that such an effect has been observed. The ability of ADPβS to exert its effects after oral administration supports the potential interest of this P_{2y}-agonist as an insulin stimulating agent.

Another potential advantage could be the ability of ADPβS to improve glucose tolerance. Our results obtained both in anaesthetized rats and in conscious dogs show that this substance, administered either intravenously or orally, enhanced the rise in plasma insulin levels induced by a glucose load and reduced the hyperglycaemia.

From this study it can be concluded that: (1) ADPβS is a potent insulin stimulating agent *in vivo* in rats and dogs; (2) ADPβS administration improves glucose tolerance; (3) ADPβS is able to act after oral administration. Thus, the P_{2y}-purinoceptors of the β cell may be a target for new antidiabetic drugs.

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