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P_{2y} purinoceptor responses of β cells and vascular bed are preserved in diabetic rat pancreas

[1]D. Hillaire-Buys, R. Gross, J. Chapal, G. Ribes & M.M. Loubatières-Mariani

Faculté de Médecine, Laboratoire de Pharmacologie, CNRS, URA 599, Montpellier Cedex, France

1 To investigate the effect of experimental diabetes on the P_{2y} purinoceptor responses of pancreatic β-cells and vascular bed, we used adenosine-5'-O-(2-thiodiphosphate) (ADPβS), a potent and stable P_{2y} agonist. This work was performed in the isolated perfused pancreas of the rat.

2 Diabetes was induced by streptozotocin (66 mg kg^{-1}, i.p.). Five weeks after the induction of diabetes, on the day of pancreas isolation, the animals displayed marked hyperglycaemia (37.6 ± 2.7 mM). Age-matched rats were used as controls.

3 Insulin response to a glucose stimulation from 5 to 10 mM was completely lost and stimulation of insulin release by the sulphonylurea, tolbutamide (185 μM), was drastically impaired in the diabetic pancreas (maximum responses were 1.5 ± 0.4 and 7.0 ± 1.4 ng min^{-1} for diabetic and age-matched rats respectively).

4 In contrast, in the diabetic pancreas ADPβS (15 μM), infused in the presence of glucose 5 mM, elicited an immediate and significant insulin release similar to that observed in the age-matched pancreas (maximum responses were 7.6 ± 1.5 and 6.7 ± 1.3 ng min^{-1} respectively). This ADPβS stimulating effect occurred independently of the glucose concentration (5, 8.3 and 28 mM) in the diabetic pancreas. On pancreatic vascular resistance, ADPβS induced a similar vasodilatation in diabetic and age-matched rats.

5 In conclusion, ADPβS retains its insulin stimulatory and vasodilator effects in experimental diabetes; P_{2y} purinoceptors could therefore be considered as a new target for the development of antidiabetic drugs.

Keywords: P_{2y} purinoceptors; adenosine-5'-O-(2-thiodiphosphate) (ADPβS); streptozotocin; diabetic rats; insulin; pancreatic vessels

Introduction

The impairment of insulin secretion in non insulin-dependent diabetes mellitus has been extensively studied; in various models of experimental diabetes, insulin response to glucose is lost whereas that to other secretagogues, such as arginine and carbachol, appears to be less affected, preserved or even increased depending on the experimental conditions (Weir *et al.*, 1981; Giroix *et al.*, 1983; Okabayashi *et al.*, 1989). In addition, vascular dysfunctions altering the responses to various vasodilator or vasoconstrictor agents develop during the diabetic state (Kamata *et al.*, 1989a,b; Mulhern & Docherty, 1989).

Purine nucleotides or nucleosides are widely known to affect many cellular processes via two types of purinoceptors: P_1 receptors for adenosine and P_2 receptors for ATP and/or ADP (Burnstock, 1978; Gordon, 1986; White, 1988). Concerning pancreatic endocrine function, we have shown that A cells possess P_1 and β cells P_2 receptors, the stimulation of which evokes glucagon and insulin secretion respectively (Loubatières-Mariani & Chapal, 1988). Adenosine receptors on A cells were shown to belong to the A_2 subtype. P_2 purinoceptors have also been subclassified into P_{2x} and P_{2y} subtypes (Burnstock & Kennedy, 1985). A previous study has demonstrated that the purinoceptors of the pancreatic β cells were of the P_{2y} subtype (Bertrand *et al.*, 1987). In addition, it has been recently shown in rat isolated perfused pancreas that adenosine-5'-O-(2-thiodiphosphate) (ADPβS), a stable P_{2y} purinoceptor agonist, elicited both insulin release and vasodilatation (Bertrand *et al.*, 1991) via the activation of P_{2y} receptors on β cells and vessels; this substance was about 100 times more potent than ATP. In pancreas from rats with streptozotocin-induced diabetes (STZ-D), we have shown that P_1-mediated glucagon secretion and blood vessel dilatation responses to adenosine were abolished and strongly reduced respectively (Gross *et al.*, 1989). In the present study we wished to obtain further insight into possible other diabetes-induced disturbances of the purinergic control of pancreatic endocrine and vascular functions. We therefore investigated whether STZ-D could modify the insulin response as well as the vasodilatation evoked by the stable agonist ADPβS via activation of P_{2y} purinoceptors.

Methods

Animals

Our study was carried out with adult male Wistar rats that had free access to food and water. All rats were housed in individual cages; a 10-day acclimatization period was allowed before the experiments began; the rats weighed from 320 to 350 g. Diabetes was induced by an i.p. injection of streptozotocin (66 mg kg^{-1}). A group of normal rats served as age-matched controls. Thereafter, daily glucosuria measurements were made to ensure the diabetic state of the animals over 5 wk; their glucosuria was higher than 10 g per 24 h. On the day of the experiment, just before the pancreas was removed, blood was sampled by intracardiac puncture for measurement of glycaemia and plasma insulin and glucagon concentrations.

Pancreas isolation

Animals were anaesthetized with 60 mg kg^{-1} i.p. pentobartitone sodium. The procedure of Loubatières *et al.* (1969) was used to isolate the pancreas completely from all neighbouring tissues. The pancreas was then transferred into a

[1] Author for correspondence at: Laboratoire de Pharmacologie, Faculté de Médecine, Institut de Biologie, Boulevard Henri IV, 34060 Montpellier Cedex, France.

plastic chamber maintained at 37.5°C. The perfusion medium, which was not allowed to recirculate, was Krebs-Ringer buffer containing 2 g l^{-1} bovine serum albumin (fraction V, Sigma, St. Louis, MO, U.S.A.) and 5 mM glucose, and was continuously bubbled with 95% O_2/5% CO_2 to maintain the pH between 7.35 and 7.40.

A peristaltic pump was used for the circulation of the perfusion medium. Perfusion pressure was maintained constant, and the excess of medium not accepted by the organ at the given pressure, returned to the origin reservoir. The pressure was selected to provide a basal vascular flow rate of about 2.5 ml min^{-1} at the beginning of the experiment. Any change in pancreatic vascular bed resistance due to drug administration resulted in changes in outflow rate; the latter was measured by collecting outflow samples in graduated tubes during 1 min.

For determination of insulin concentration, a 1 ml aliquot of pancreatic effluent was immediately frozen. For determination of glucagon concentration, a 500 µl aliquot of pancreatic outflow was collected in chilled tubes containing 50 µl of a mixture of 32 mM EDTA and 10,000 KIU ml^{-1} aprotinin (Antagosan, Hoechst, Puteaux) and immediately frozen.

Experimental protocol

The first sample was taken after a 30 min equilibration period (time measured from beginning of pancreas perfusion). Another sample was collected 15 min later. Drugs, ADPβS (Boehringer, Mannheim, FRG) or tolbutamide (Sigma, St. Louis, MO, U.S.A.), were then administered for 30 min, and samples were collected every min for 5 min and then, 8, 10, 15 and 30 min after the beginning of drug infusion. The effects were studied simultaneously in age-matched and diabetic rats.

In a separate set of experiments, we investigated the effect of 5-wk diabetes on glucagon and insulin contents in islets. Batches of 10 islets were prepared, from normal and diabetic rat pancreas, according to the isolation technique of Lacy & Kostianowski (1967); for the extraction of peptides, they were incubated for 24 h at 4°C in 1 ml acid ethanol (pure HCl and 75% ethanol (1:65 vol/vol)).

Assay methods

Blood glucose and glucosuria were measured by the potassium ferricyanide method with a Technicon autoanalyser (Alric *et al.*, 1965). Insulin concentration was determined according to the method of Herbert *et al.* (1965). We used rat insulin as a standard and the anti-insulin serum supplied by Miles Laboratories (Paris). The sensitivity of the method was 0.1 ng ml^{-1}. Glucagon concentration was measured according to the radioimmunological method described by Unger *et al.* (1970). We used the BR 124 antiglucagon serum supplied by the Institut de Biochimie Clinique of Geneva. The sensitivity of the method was 15 pg ml^{-1}. Results are given as picogram equivalents of porcine glucagon.

Data analysis

Results are given as mean ± s.e.mean. Insulin and glucagon outputs were calculated by multiplying the hormone concentration (ng ml^{-1} or pg ml^{-1}) by the vascular flow rate (ml min^{-1}). Insulin and glucagon outputs are expressed as absolute values; vascular outflow rate is expressed as a percentage of the 45 min value (100%). Kinetic data were submitted to analysis of variance with the multiple-comparison test (Zar, 1974).

Results

Characteristics of diabetic state

In Table 1 are shown various parameters recorded just before removal of the pancreas, 5 wk after STZ injection. Diabetic animals were markedly hyperglycaemic and glucosuric and their mean body weight had not increased. They were strongly hypoinsulinaemic and the determination of islet insulin storage showed that insulin content was drastically decreased.

Insulin response

We first tested the effect of a glucose infusion and of an insulin secretory agent, tolbutamide. Basal insulin secretion with a non-stimulatory glucose concentration (5 mM) was similar in normal and diabetic rats (0.8 ± 0.1 and 0.7 ± 0.1 ng min^{-1} respectively). Increasing glucose concentration from 5 to 10 mM elicited an immediate and biphasic insulin response in age-matched rats ($P<0.001$), whereas the insulin response was totally abolished in diabetic rats (Figure 1). The insulin response to the sulphonylurea, tolbutamide (185 µM), in the presence of 5 mM glucose was drastically impaired in diabetic rats (Figure 2). The maximum insulin release observed was 7.0 ± 1.4 ($P<0.001$) and 1.5 ± 0.4 ng min^{-1} ($P<0.05$) for age-matched and diabetic rats respectively (respective basal values being 1.0 ± 0.3 and 0.6 ± 0.2 ng min^{-1}). Insulin output was stable throughout the experiment in the presence of glucose (5 mM) alone in age-matched and diabetic rats (Figure 3).

ADPβS (15 µM) infused in the presence of 5 mM glucose increased insulin secretion both in age-matched and diabetic animals (Figure 3). The insulin stimulating effect of ADPβS occurred immediately and insulin release peaked at 6.7 ± 1.3 ($P<0.001$) and 7.6 ± 1.5 ng min^{-1} ($P<0.001$) in age-matched and diabetic rats respectively (basal values being 1.0 ± 0.1 and 0.8 ± 0.2 ng min^{-1}). Thereafter insulin secretion remained slightly higher than the basal values in both groups. The kinetics of insulin output was similar in age-matched and diabetic rats. In the diabetic pancreas, ADPβS was then tested in the presence of different glucose concentrations: 8.3 and 28 mM. The stimulatory effects on insulin secretion were comparable to those obtained in the presence

Table 1 Various parameters recorded in non-fasting diabetic or normal rats just before pancreas extirpation: islet insulin and glucagon contents obtained in a separate set of experiments are given

	Body weight (g)	*Glucosuria* (g per 24 h)	*Glycaemia* (mM)	*Plasma insulin* (ng ml^{-1})	*Plasma glucagon* (pg ml^{-1})	*Islet insulin content* (ng per islet)	*Islet glucagon content* (pg per islet)
Age-matched rats	471	0	9.6	2.80	144	27.7	1217
	± 8		± 0.3	± 0.30	± 7	± 5.8	± 266
Diabetic rats	309	14.1	37.6	0.30	297	3.0	1609
	± 10	± 1.0	± 2.7	± 0.05	± 15	± 0.5	± 447

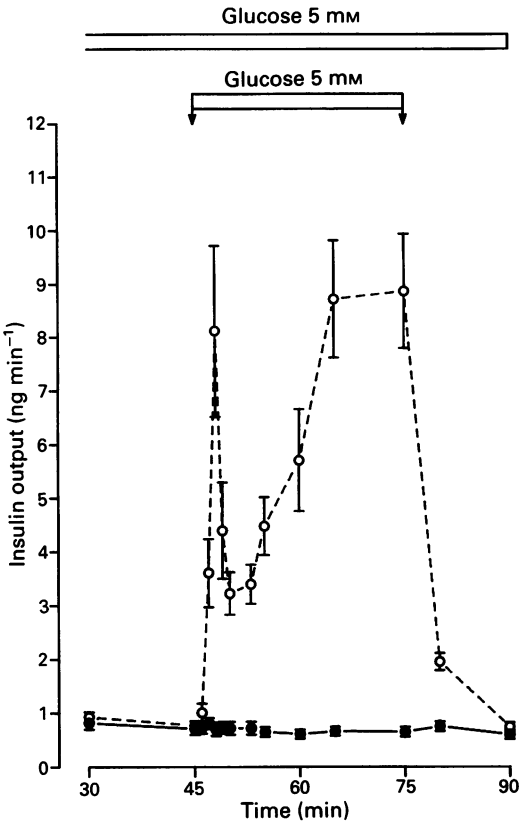

Figure 1 Insulin secretion in response to an increasing glucose concentration (5 to 10 mM), from the isolated perfused pancreas from 5-wk streptozotocin-induced diabetic (●) and age-matched (○) rats. Each point represents the mean with s.e.mean shown by vertical bars from 6 experiments.

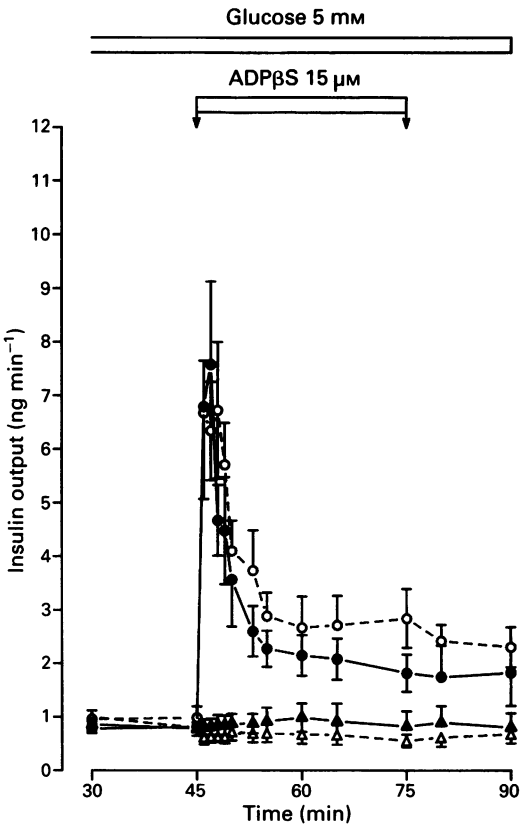

Figure 3 Insulin secretion in response to adenosine-5'-O-(2-thiodiphosphate) (ADPβS, 15 μM) from isolated perfused pancreas from 5-wk streptozotocin-induced diabetic (●) and age-matched (○) rats. Controls perfused with glucose 5 mM alone are shown: age-matched (△) and diabetic (▲) rats. Each point represents the mean with s.e.mean shown by vertical bars from 6 experiments.

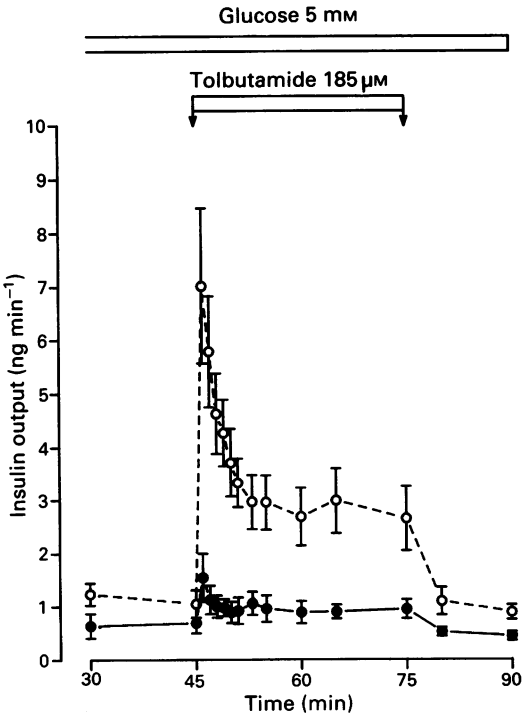

Figure 2 Insulin secretion in response to tolbutamide (185 μM) from isolated perfused pancreas from 5-wk streptozotocin-induced diabetic (●) and age-matched (○) rats. Each point represents the mean with s.e.mean shown by vertical bars from 6 experiments.

of 5 mM glucose (Figure 4) (the maximum insulin release being 6.1 ± 1.6 and 9.1 ± 1.2 ng min^{-1} respectively for diabetic pancreas perfused with 8.3 or 28 mM glucose).

Glucagon response (Figure 5)

Under basal conditions, in the presence of glucose 5 mM, glucagon output was stable during the whole experiment in normal as well as in diabetic rats. However, it was observed that the basal glucagon release was lower in diabetic than in normal rats. ADPβS (15 μM) caused an immediate but slight and transient rise in glucagon output ($P < 0.01$ versus 45 min value within 2 min of infusion) in age-matched rat pancreas. This effect of ADPβS was not observed in 5 wk diabetic rats.

Vascular response

In control experiments performed in the presence of glucose 5 mM alone, the vascular flow rate was not significantly modified during the entire 90 min experiment, although there was a very slight and progressive but not significant decrease to 97 ± 2 and 96 ± 4% of the 45 min values, which were 2.43 ± 0.04 and 2.49 ± 0.03 ml min^{-1} for age-matched and diabetic rats respectively. ADPβS (15 μM) induced a dual effect on pancreatic vascular flow rate in age-matched rats: a first transient vasoconstriction, significant only at the second min, ($P < 0.01$ versus 45 min value) followed by a long lasting and sustained vasodilatation (Figure 6). In the diabetic rat pancreas, an immediate and significant vasodilatation was observed which persisted throughout the ADPβS infusion ($P < 0.001$ versus 45 min value). From 65 min on, the maximum vasodilator effect was similar in age-matched and diabetic rats (+ 16 ± 4 and + 16 ± 3% respectively).

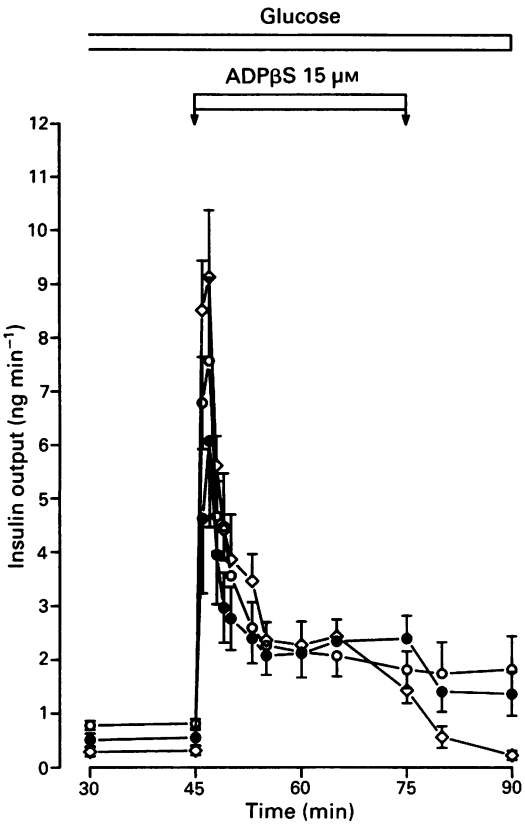

Figure 4 Effects of adenosine-5'-O-(2-thiodiphosphate) (ADPβS, 15 μM) on insulin secretion from isolated perfused pancreas from 5-wk streptozotocin-induced diabetic rats in presence of different glucose concentrations: (○) 5 mM; (●) 8.3 mM; and (◇) 28 mM glucose. Each point represents the mean with s.e.mean shown by vertical bars from 6 experiments.

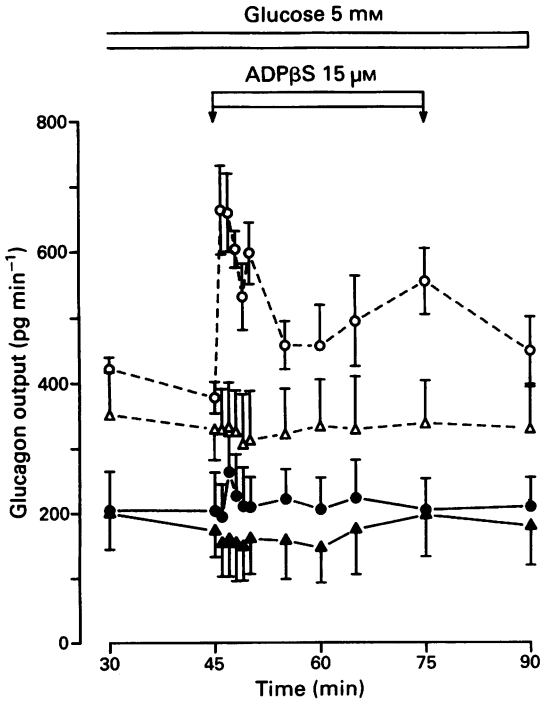

Figure 5 Glucagon secretion in response to adenosine-5'-O-(2-thiodiphosphate) (ADPβS, 15 μM) from isolated perfused pancreas from 5-wk streptozotocin-induced diabetic (●) and age-matched (○) rats. Controls perfused with glucose 5 mM alone are shown: age-matched (△) and diabetic (▲) rats. Each point represents the mean with s.e.mean shown by vertical bars from 6 experiments.

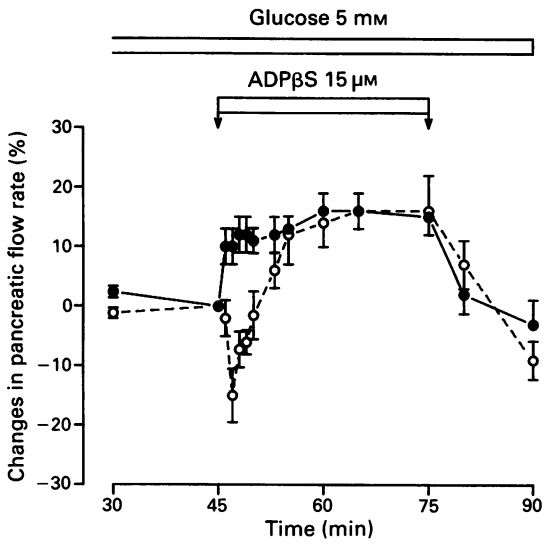

Figure 6 Effects of adenosine-5'-O-(2-thiodiphosphate) (ADPβS, 15 μM) on vascular flow rate in isolated perfused pancreas from 5-wk streptozotocin-induced diabetic (●) and age-matched (○) rats in presence of 5 mM glucose. Each point represents the mean with s.e.mean shown by vertical bars from 6 experiments.

Discussion

Our results show that (1) the pancreas from adult STZ-diabetic rats with impaired responses to glucose and tolbutamide, retain their ability to secrete insulin under ADPβS stimulation; (2) ADPβS remains fully active in inducing vasodilatation in diabetic rats.

The glucose insensitivity of the diabetic pancreas that we observed in our model of STZ-D rats, is in total agreement with other data obtained with animals rendered diabetic by STZ in lower dosage (Okabayashi *et al.*, 1989) or as neonates (Weir *et al.*, 1981; Giroix *et al.*, 1983). The same results were reported in spontaneously diabetic BB rats (Grill & Herberg, 1983) where the β cells were not destroyed by diabetogenic substances like STZ or alloxan, but by immunological factors (Marliss *et al.*, 1982). In another model with reduced β cell mass by partial pancreatectomy (pancreatic remnant being approximately 12% of the whole pancreas), a poor but significant response to glucose was preserved (Leahy *et al.*, 1984).

The most striking observation of the present study is that the remaining β cells of this diabetic rat model are extremely sensitive to ADPβS, since we obtained a comparable response in the age-matched and diabetic pancreas, despite a 10 times lower pancreatic insulin content in the latter. In our study, this P_{2y} purinoceptor agonist evoked a similar pattern of insulin response in the presence of higher glucose concentrations, 8.3 and 28 mM (the latter concentration mimicking glycaemia observed in these diabetic rats), thereby confirming β cell insensitivity to glucose in the diabetic pancreas. The results obtained with ADPβS contrast with those obtained with the antidiabetic sulphonylurea, tolbutamide, the insulin secretory effect of which was drastically decreased in our diabetic model. This finding is in agreement with the work of Weir *et al.* (1981) showing that tolbutamide, at the same concentration, was ineffective in eliciting an insulin response in neonatal STZ-D rats in the presence of glucose 7.8 mM; however, it is at variance with other data obtained in the same diabetic model, but in the absence of glucose (Giroix *et al.*, 1983; Serradas *et al.*, 1989). It is noteworthy that, in addition to their responsiveness to cholinergic, β-adrenergic and amino acid stimulation (Weir *et al.*, 1981; Giroix *et al.*, 1983; Grill & Herberg, 1983; Okabayashi *et al.*, 1989), diabetic β cells retain their ability to respond to purinergic stimulation.

Confirming our previous study (Gross *et al.*, 1989) basal *in vitro* glucagon output was found reduced in diabetic pancreas; this contrasted with the marked hyperglucagonaemia that occurred *in vivo*, suggesting that the high plasma glucagon levels measured originate from extrapancreatic sources. ADPβS elicited a slight and transient response of A cells in the age-matched rat. The rapidity of onset and transient nature of the glucagon response to ADPβS is in favour of an action via a P_1 site (on A cells) rather than a breakdown of this stable compound into adenosine. Such an action of ADPβS on a P_1 site has been described in rabbit jugular vein (Wood *et al.*, 1989). This glucagon response to ADPβS was completely suppressed in 5-wk-diabetic rats and is in agreement with our previous study showing that the A cell response to P_1 receptor activation was abolished in the diabetic state (Gross *et al.*, 1989).

Concerning our data on pancreatic vascular resistance in the diabetic state, ADPβS induced only a relaxing effect which contrasts with the biphasic response observed in age-matched rats: a transient vasoconstriction followed by a vasodilatation. The biphasic pattern of pancreatic vascular bed response obtained in our control group, could be explained by the existence of 2 types of receptors previously shown to regulate pancreatic tone (Hillaire-Buys *et al.*, 1991): P_{2y} and P_{2x} purinoceptors inducing vasodilator and vasoconstrictor effects respectively. As the contractile properties of rat aorta and mesenteric artery are in part dependent upon the smooth muscle mass of the preparation (Cohen & Berkowitz, 1974), ADPβS failure to induce vasoconstriction in diabetic rats could be ascribed to diabetes-induced reduction of the mass of smooth muscle. With this in mind, we performed a set of experiments with weight-matched (but not age-matched) rats, and the vasoconstrictor effect of ADPβS could no longer be observed (data not shown) confirming previous results obtained in normal rats weighing about 330–350 g (Bertrand *et al.*, 1991). In this regard, it has been reported that the adrenergic contractile response of aortic and mesenteric artery strips is age-dependent (Cohen & Berkowitz, 1974); our results with ADPβS in normal rats may suggest that the purinoceptor-induced constrictor effect in pancreatic vascular bed could also be age-dependent. An alternative explanation for the failure of ADPβS to induce vasoconstriction in diabetic rats could be the impairment of P_{2x} receptors by experimental diabetes. This is unlikely since the P_{2x} selective agonist, α,β-methylene ATP, induced similar transient vasoconstrictor responses both in diabetic and age-matched rats (respective maximal decrease of vascular flow rate reaching -60 ± 8 and $-69 \pm 5\%$ at 2 min with 0.5 μM α, β-methylene ATP). Whatever it may be, it is noteworthy that, the vasodilator effect due to P_{2y} purinoceptors is preserved and prevails in diabetic rats. These data are interesting when compared with our previous studies which showed that in diabetic rats, vasodilator responses to adenosine, isoprenaline and forskolin were decreased (Gross *et al.*, 1989; 1991), suggesting an impairment of the adenylate cyclase pathway. Thus the retained ability of ADPβS to induce a vasodilator response in diabetic rats suggests that the mechanisms underlying the effect of the P_{2y} agonist are unaffected by the diabetic state.

In conclusion, ADPβS retains its insulin stimulatory and vasodilator effects in experimental diabetes; P_{2y} purinoceptors should therefore be considered as a new target for the development of antidiabetic drugs.

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