P2Y Purinergic Potentiation of Glucose-Induced Insulin Secretion and Pancreatic β-Cell Metabolism

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Purine nucleotides and their analogs increase insulin secretion through activation of pancreatic β-cell P2Y receptors. The present study aimed at determining the role of glucose metabolism in the response to P2Y agonists and whether ATP-activated K^+ channels (K_{ATP} channels) are involved in this response. The experiments were performed in the rat isolated pancreas, perfused with a Krebs-bicarbonate buffer supplemented with 2 g/l bovine serum albumin under dynamic glucose conditions from 5 mmol/l baseline to 11 mmol/l. ADPβS (0.5 μmol/l) was selected as a stable and selective P2Y agonist. This compound, ineffective on the 5 mmol/l glucose background, induced a significant threefold increase in insulin release triggered by the glucose challenge. The effect of ADPβS was markedly reduced ($P < 0.001$) in the presence of an inhibitor of glucose metabolism. In addition to glucose, the ADP analog also amplified the β-cell insulin response to 15 mmol/l methyl pyruvate ($P < 0.05$), but it was ineffective on the insulin response to 2.5 mmol/l methyl succinate. A nonmetabolic stimulus was applied using tolbutamide (185 μmol/l). Insulin secretion induced by the K_{ATP} channel blocker was strongly reinforced by ADPβS ($P < 0.001$), which prompted us to check a possible interplay of K_{ATP} channels in the effect of ADPβS. In the presence of diazoxide 250 μmol/l and 21 mmol/l KCl, ADPβS still amplified the second phase of glucose-induced insulin secretion ($P < 0.001$). We conclude that P2Y receptor activation is able to promote insulin secretion through a mechanism, involving β-cell metabolism and a rise in intracellular calcium; this effect does not result from a direct inhibitory effect on K_{ATP} channels. *Diabetes* 53 (Suppl. 3):S63–S66, 2004

Purine nucleotides (ATP and ADP) and their analogs have been shown to increase glucose-induced insulin secretion through activation of P2 receptors present on pancreatic β-cells (1). These receptors were characterized as P2Y receptor subtypes (2), of which the structural analog ADPβS [adenosine-5′-*O*-(2-thiodiphosphate)] is a stable and potent agonist (3). The pharmacological properties of β-cell P2 receptors have been reviewed elsewhere (4).

The activation of P2Y receptors in vivo leads to an insulin response that is clearly dependent on the nutritional status of the animals and the plasma level of glucose; this action improves glucose tolerance (5). The glucose dependency of the insulin response brought about by P2Y receptor activation was also shown in vitro in rat (6) as well as in human islets (7). The stimulus-secretion coupling of β-cell P2 receptors is still a matter of investigation. Both an increased influx of extracellular calcium (8) and an intracellular calcium mobilization (9) have been reported. An increase in cytoplasmic free calcium concentration has been consistently observed (10). In human pancreatic islets and isolated human insulin-secreting cells, extracellular ATP was also shown to raise the cytoplasmic free Ca^{2+} concentration (11). In addition, alterations in membrane potassium conductance by extracellular ATP or structural analogs have also been described in mouse islets and single β-cells (12), as well as in RINm5F insulinoma cells (13). A slight decrease in the membrane potassium conductance has been reported in rat islets with a P2X but not with a P2Y agonist (6), and a P2X receptor subtype has been characterized, with functional activity at a low, nonstimulating glucose concentration. More recently, Chevassus et al. (14) showed that a P2Y receptor activation amplifies glucose-induced insulin secretion by activating β-cell adenylyl cyclase and the subsequent cAMP/protein kinase A signaling pathway in rat pancreatic islets and in the INS-1 secreting cell line.

The present study was designed to further investigate the mechanism whereby P2Y receptor activation amplifies insulin response in dynamic conditions that mimic a postprandial glucose challenge.

RESEARCH DESIGN AND METHODS

Experimental procedure. Experiments were performed in vitro in the isolated perfused pancreas from male Wistar albino rats (Charles River, Lyon, France). Rats weighing 340–360 g were housed in a light-controlled room and given free access to food and water in accordance with the national guidelines for the use and care of laboratory animals. After sodium pentobarbitone anesthesia (60 mg/kg i.p.), the pancreas was completely isolated from all the neighboring tissues, according to a technique previously described (15), and perfused in the presence of a 5 mmol/l glucose background. A 30-min adaptation period was allowed before the first sample was taken for insulin assay and was followed by a further 30-min control period before any change in glucose concentration, or in type of secretagogue, and ADPβS administration. Upon administration of various secretagogues in the presence or absence of ADPβS, pancreatic effluents were collected every minute during the first 5 min immediately after the beginning of the treatments. Additional samplings were performed after 10, 15, and 20 min. Effluents were measured in graduated test tubes and immediately frozen until insulin assay.

Chemicals. ADPβS [adenosine 5′-*O*-(2-thiodiphosphate)], glucose, sodium nitroprusside, tolbutamide, pyruvic acid methyl ester, succinic acid mono-

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ADPβS, adenosine 5′-*O*-(2-thiodiphosphate); K_{ATP} channel, ATP-activated K^+ channel.

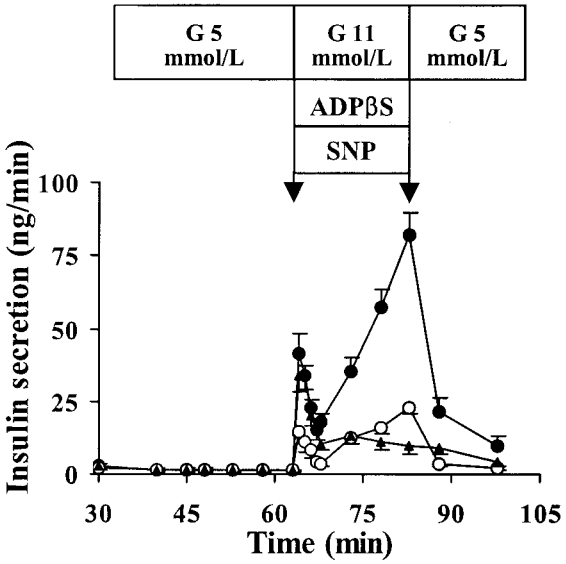

FIG. 1. Insulin release induced by a glucose challenge (from 5 to 11 mmol/l) in the absence (○) and in the presence (●) of ADPβS (0.5 μmol/l). Effects of SNP (300 μmol/l) (▲) on insulin release in the presence of ADPβS (0.5 μmol/l). The results are the means ± SE of seven experiments. G, glucose.

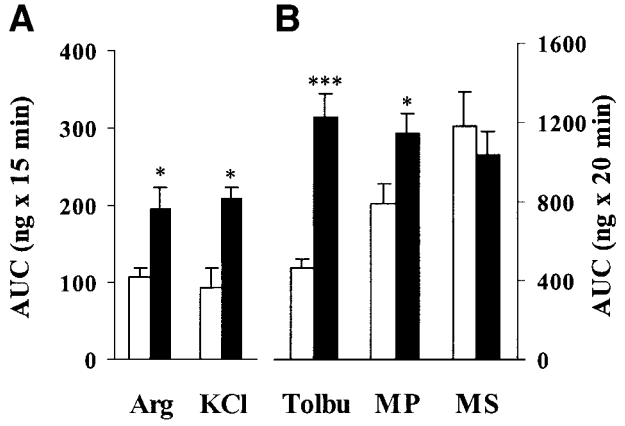

FIG. 2. AUCs during the last 15 min (*A*) or the whole 20 min (*B*) of insulin secretion induced by different secretagogue agents: arginine (Arg, 5 mmol/l), KCl (5 mmol/l), tolbutamide (Tolbu, 185 μmol/l), methyl pyruvate (MP, 15 mmol/l), and methyl succinate (MS, 5 mmol/l) in the absence (□) or in the presence (■) of ADPβS (0.5 μmol/l). The results are the means ± SE of 6–8 experiments (*$P < 0.001$; *$P < 0.05$). Please note the two different scales on *y*-axis of panels *A* and *B*.**

methyl ester, arginine hydrochloride, bovine serum albumin (fraction V), and diazoxide were purchased from Sigma Aldrich (St. Louis, MO). Other chemicals were obtained from various commercial sources at the greatest purity available.

Assays. Insulin concentrations were determined by the radioimmunological method of Herbert et al. (16) using purified rat insulin as standard (Linco Research, St. Charles, MO) and guinea pig antiporcine-insulin antiserum (ICN Biochemicals, Puteaux, France). The sensitivity of our assay was 0.06 ng/ml.

Data expression and statistical analysis. Insulin output was calculated by multiplying concentration in the effluent by pancreatic outflow rate measured during 1 min. Results are expressed as means ± SE. Integrated data were obtained by calculating the areas under the curves during the 20 or last 15 min (for arginine and KCl) of high glucose or of other secretagogue administration. Kinetics data were submitted to ANOVA followed by the multiple comparison test of Newman-Keuls and integrated data by the Student's *t* test.

RESULTS

Potentiation of glucose-induced insulin secretion by ADPβS and effect of an inhibitor of glucose metabolism. ADPβS (0.5 μmol/l), ineffective on the 5 mmol/l glucose background (data not shown), causes a threefold increase in insulin release induced by a rise in glucose concentration from 5 to 11 mmol/l (Fig. 1). The typical biphasic pattern of insulin response to glucose was retained with a mean integrated response reaching 845 ± 83 ng × 20 min vs. 251 ± 19 ng × 20 min in the absence of ADPβS ($P < 0.001$). As shown also in Fig. 1, the nitric oxide donor sodium nitroprusside (SNP) at a concentration of 300 μmol/l is able to drastically reduce the second phase of secretion in the presence of the P2Y receptor agonist; the last 15 min integrated response was reduced by 75%, reaching 171 ± 36 ng × 15 min vs. 678 ± 71 ng × 15 min in the absence of the metabolic inhibitor ($P < 0.001$).

Nonmetabolic β-cell depolarization and potentiation of insulin secretion by ADPβS. A 5 mmol/l increase in the concentration of the biophysical depolarizing agent KCl provoked a biphasic response of insulin secretion. After a 5-min first phase, insulin secretion stabilized slightly over basal values. Addition of ADPβS resulted in the potentiation of the second phase (Fig. 2) with a 15-min area under the curve (AUC) reaching 210 ± 14 ng × 15 min vs. 93 ± 26 ng × 15 min in the absence of ADPβS ($P < 0.05$). Similar data could be observed with arginine as a depolarizing agent. When tolbutamide (185 μmol/l) was used as a nonmetabolic stimulus, β-cell membrane depolarization resulted also in a biphasic insulin response in the presence of the 5 mmol/l glucose concentration. Simultaneous administration of ADPβS markedly potentiated both phases of tolbutamide effect. As shown in Fig. 2, mean 20 min integrated insulin secretion reached 1,229 ± 121 ng × 20 min and 462 ± 51 ng × 20 min in the presence and the absence, respectively, of ADPβS ($P < 0.001$).

ADPβS and insulin secretion induced by pyruvate and succinate methyl esters. Methyl pyruvate (15 mmol/l) induced a biphasic insulin response with a 20-min AUC reaching 791 ± 102 ng × 20 min. Addition of ADPβS resulted in a slight but significant 45% increase (1,145 ± 99 ng × 20 min; $P < 0.05$; Fig. 2). In contrast, when methyl succinate (2.5 mmol/l) was used as a secretagogue, no further increase could be observed in the presence of the P2Y receptor agonist. Mean integrated data reached 603 ± 85 ng × 20 min and 681 ± 90 ng × 20 min in the presence and absence, respectively, of ADPβS. Similar data could be observed in the presence of 5 mmol/l methyl succinate (Fig. 2).

Effect of ADPβS (0.5 μmol/l) on glucose-induced insulin secretion in the presence of diazoxide 250 μmol/l and 21 mmol/l KCl. Administration of diazoxide and KCl, during a 15-min pretreatment on the 5 mmol/l glucose background, induced a slight increase in insulin secretion. Raising glucose concentration from 5 to 11 mmol/l provoked an increase in insulin secretion with a mean 20-min AUC reaching 366 ± 52 ng × 20 min (Fig. 3*A*). It is noteworthy that the first phase of insulin response was completely suppressed. Upon addition of ADPβS, β-cell response appeared markedly enhanced by 200%. It must be mentioned that the increase observed during the first 5 min is not due to restoration of a first phase since it also occurs with ADPβS in the absence of any change in glucose concentration (Fig. 3*B*). It may be ascribable to the potentation by ADPβS of the effect of a

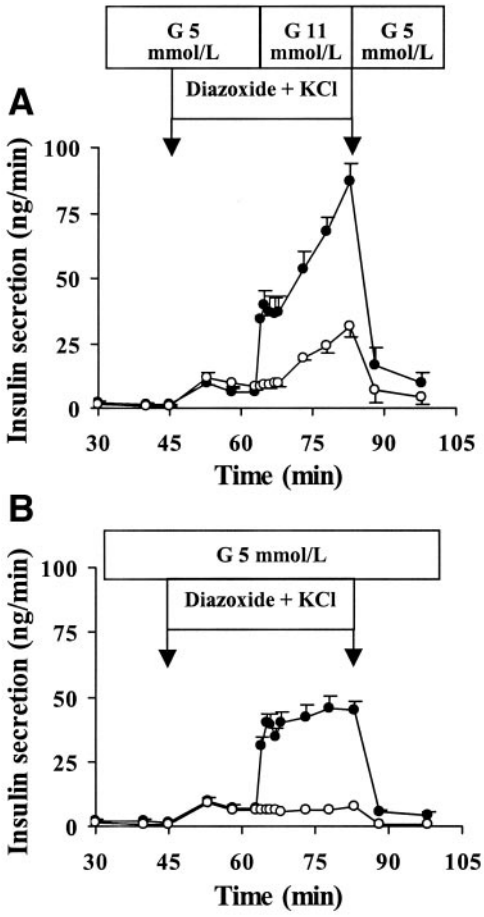

FIG. 3. Insulin release in the presence of diazoxide (250 μmol/l) and KCl (21 mmol/l), in the absence (○) or in the presence (●) of ADPβS (0.5 μmol/l) during a rise in glucose concentration (*A*) or on the 5 mmol/l glucose background (*B*). The results are the means ± SE of eight experiments. G, glucose.

slight increase in intracellular Ca^{2+} during the diazoxide-KCl pretreatment, which also accounts for the moderate elevation of insulin secretion over basal values.

DISCUSSION

Our data demonstrate that the stable P2Y receptor agonist ADPβS potentiates glucose-induced insulin secretion during an acute glucose challenge that mimics a postprandial rise in the sugar concentration. This effect depends on glucose metabolism, is independent of a direct effect on K_{ATP} channels, but probably requires a rise in intracellular Ca^{2+} concentration. The involvement of glucose metabolism is clearly demonstrated by our experiments performed in the presence of SNP, a chemical NO donor. Indeed, NO has been reported to inhibit important glycolytic enzymes such as phosphofructokinase (17) and glyceraldehyde phosphate dehydrogenase (18) as well as mitochondrial cytochrome oxidase (19), and the NO donor drastically decreases the ADPβS-potentiating effect. Because glucose is known to trigger insulin secretion through its oxidative metabolism, rise in the ATP/ADP ratio, closure of K_{ATP} channels, membrane depolarization, opening of voltage-operated Ca^{2+} channels, Ca^{2+} influx, rise in cytoplasmic free Ca^{2+} concentration, and activation of the exocytotic machinery (20), we wondered whether ADPβS could be able to potentiate insulin secretion induced by a nonmetabolic membrane depolarization. Using tolbutamide, a sulfonylurea known as a K_{ATP} channel blocker, we show that the P2Y receptor agonist is still strongly effective, suggesting that a rise in cytosolic Ca^{2+} is probably involved in the potentiating effect. This is also supported by the data resulting from experiments using two other nonmetabolic depolarizing agents, KCl and arginine, but raises the question of a possible direct involvement of K_{ATP} channels. To investigate such a possibility, we first adapted the elegant method developed by Gembal et al. (21) to the "isolated perfused rat pancreas" model. Therefore, pancreatic β-cells were first hyperpolarized using the K_{ATP} channel opener diazoxide and then repolarized with an appropriate rise in KCl concentration. Because vascular smooth muscle cells are regulated by K_{ATP} channels (22), we used our results on pancreatic effluent output rate to determine the KCl concentration necessary to repolarize these cells (i.e., the concentration required to completely counteract the vasodilator effect of diazoxide). Under such conditions (i.e., bypass of K_{ATP} channels in the presence of clamped intracellular Ca^{2+} concentration), ADPβS still strongly potentiates the amplifying pathway of glucose-induced insulin secretion, which definitely excludes a possible direct effect on K_{ATP} channel activity in the effect of P2Y receptor activation on pancreatic β-cell function. As concerns the triggering pathway, the potentiation of glucose- and tolbutamide-induced first phases, but not of arginine- and KCl-induced first phases, raises the possibility of a modulation by the P2Y agonist, of K_{ATP} channel closure induced by either glucose or tolbutamide. Such an additional mechanism might be put together with the effect of GLP-1 on K_{ATP} channels mediated by protein kinase A (PKA) (23).

The importance of glucose metabolism prompted us to investigate the effect of ADPβS on insulin secretion induced by the cell permeant methyl esters of two tricarboxylic acid cycle intermediates: pyruvate and succinate. These compounds have both been reported to stimulate β-cell function, not exclusively through mitochondrial ATP production. Methyl pyruvate has first been proposed to initiate insulin release through an extramitochondrial effect or production of mitochondrial coupling factors different from ATP (24), and more recently through a direct inhibitory effect on K_{ATP} channels (25). In our conditions, such an action should however be of minor importance since a consistent response to the methyl ester was obtained only at the high 15 mmol/l concentration and could only be amplified by 45% in the presence of ADPβS. From the present data, we cannot conclude as to the precise mechanism involved; nevertheless, it probably differs from that of tolbutamide, which appears to be much more sensitive to the activation of P2Y receptors.

When compared with methyl pyruvate, the succinate methyl ester appears to be approximately four times more potent on insulin secretion. This probably results from the ability of succinate to react with both succinate dehydrogenase to provide metabolic energy and succinate thiokinase when present at high concentrations (26). As a consequence, succinyl CoA generated by the reversal of thiokinase reaction interacts with acetoacetate to produce succinate and acetoacetyl-CoA that would in turn react

with acetyl-CoA to yield 3-hydroxy-3-methylglutaryl-CoA, a precursor of mevalonate that has been shown to modify GTP binding proteins by isoprenylation, a prerequisite for their association to membranes (27,28). Therefore, mevalonate or one of its metabolites has been proposed as a signal for insulin secretion. If the thiokinase reaction with succinate consumes either GTP or ATP, the cost in metabolic energy is probably low in the presence of succinate esters, due to the flux of high levels of succinate through succinate dehydrogenase, which generates two molecules of ATP per molecule of succinate. Considering this succinate mechanism on insulin release proposed by Fahien and McDonald (26), the inability of ADPβS to potentiate methyl succinate–induced insulin secretion might be due to the decrease in intracellular GTP levels resulting from the reversal of succinate thiokinase reaction. Indeed, GTPase activity is well known to play a central role in the signaling pathways that couple receptors to adenylyl cyclase.

We conclude that ADPβS, a stable P2Y receptor agonist, potentiates the insulin secretory effect of a glucose challenge that mimics a postprandial increase in the sugar concentration. This effect requires glucose metabolism, is probably related to an increase in intracellular Ca^{2+} concentration, and occurs independently of a direct effect on K_{ATP} channels, although different mechanisms are probably involved for the two phases of glucose-induced insulin secretion.

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