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Cationic and secretory effects of BPDZ 44 and diazoxide in rat pancreatic islets

M.-H. Antoine, B. Pirotte[a], M. Hermann, P. de Tullio[a], J. Delarge[a], A. Herchuelz and P. Lebrun*

Laboratory of Pharmacology, School of Medicine, Brussels Free University 808, route de Lennik, B-1070 Brussels and [a]Department of Medicinal Chemistry, University of Liège (Belgium)
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Abstract. The present study aimed at comparing the effects of low concentrations of BPDZ 44, a new pyridothiadiazine derivative, and diazoxide on ^{86}Rb outflow, ^{45}Ca outflow, ^{45}Ca uptake and insulin release from rat pancreatic islets. Both drugs caused similar modifications, but the effects of BPDZ 44 on the cationic and secretory events were much more marked than those of diazoxide. It is suggested that BPDZ 44 could be valuable tool for further studies of the K_{ATP} channels.
Key words. BPDZ 44; diazoxide; $^{86}Rb/^{45}Ca$ fluxes; insulin release; rat pancreatic islets.

BPDZ 44 [3 - (1',2' - dimethylpropyl)amino - 4H - pyrido[4,3-e][1,2,4]thiadiazine 1,1-dioxide] is a newly introduced drug which is chemically related to diazoxide[1]. Recent in vitro studies reported that BPDZ 44 was more potent than diazoxide in inhibiting the glucose-induced insulin secretion from incubated rat pancreatic islets[1]. BPDZ 44 was also shown to counteract the insulin releasing process by activating the ATP-sensitive K^+ channels[1,2].

The aim of the present study was to compare the effects of low concentrations of BPDZ 44 and diazoxide on cationic and secretory events in rat pancreatic islets perfused and/or incubated in the presence of physiological concentrations of glucose. Diazoxide was selected for the purpose of comparison because this reference molecule is regarded as a potent and specific activator of the B-cell K_{ATP} channels (for reviews see 3 and 4).

Materials and methods

The methods used to measure fractional outflow rates (FOR) for ^{86}Rb (a tracer for K^+) or ^{45}Ca, ^{45}Ca uptake, and insulin release from rat pancreatic islets have been described previously[5–7]. BPDZ 44 was synthesized at the Department of Medicinal Chemistry (University of Liège, Liège, Belgium) and diazoxide was obtained from Essex Labo (Brussels, Belgium). BPDZ 44 and diazoxide were dissolved in dimethylsulfoxide which was added to both control and test media at final concentrations not exceeding 0.1% (v/v). All results are expressed as the mean ($\pm$SE) together with the number of individual experiments. The magnitude of the increase in ^{45}Ca outflow and insulin release was estimated in each individual experiment from the integrated outflow of ^{45}Ca or from the integrated output of insulin observed during stimulation (min 45–68) after correction for basal value (min 40–44). The statistical significance of differences between mean data was assessed by using Student's t-test or by analysis of variance followed for multiple comparisons by a Bonferroni test procedure.

Results

A sudden rise in the glucose concentration from 5.6 to 16.7 mM provoked a modest though sustained and rapidly reversible reduction in ^{86}Rb outflow (fig. 1, upper panel). Diazoxide (10 μM) and BPDZ 44 (10 μM), when present throughout the perfusion period, increased the basal rate of ^{86}Rb outflow recorded before the administration of 16.7 mM glucose (fig. 1 upper panel). The basal ^{86}Rb outflow (min 40–44) averaged 2.26 ± 0.06%/min in the absence; 2.69 ± 0.12%/min in the presence of diazoxide ($p < 0.01$) and 3.41 ± 0.10%/min in the presence of BPDZ 44 ($p < 0.001$). In islets exposed to diazoxide or BPDZ 44, the glucose-induced reduction in ^{86}Rb outflow persisted.

The rise in glucose concentration from 5.6 to 16.7 mM also provoked a marked increase in ^{45}Ca outflow and a biphasic insulin output (fig. 1, middle and lower panels). When the same experiment was repeated in the presence of BPDZ 44 (10 μM) throughout, the stimulatory effects of 16.7 mM glucose were completely abolished (fig. 1, middle and lower panels). In the presence of diazoxide (10 μM), raising the concentration of glucose still elicited an increase in ^{45}Ca outflow and insulin release (fig. 1, middle and lower panels). However, the increment in ^{45}Ca outflow evoked by glucose averaged 0.45 ± 0.02%/min ($n = 9$) in the absence and 0.26 ± 0.06%/min ($n = 4$) in the presence of diazoxide ($p < 0.005$). The integrated output of insulin measured during exposure to 16.7 mM glucose averaged 1.01 ± 0.10 μU · min^{-1} · islet^{-1} in the absence and 0.46 ± 0.10 μU · min^{-1} · islet^{-1} in the presence of diazoxide ($p < 0.01$).

When either BPDZ 44 (10 μM) or diazoxide (10 μM) were administered to islets perifused in the presence of 16.7 mM glucose throughout, they provoked a rapid

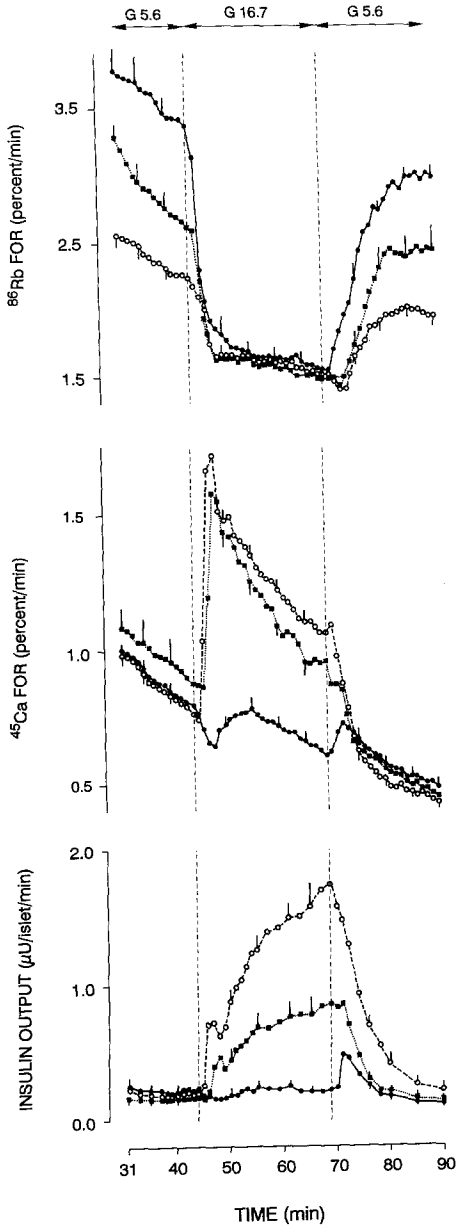

Figure 1. Effect of a rise in the glucose concentration from 5.6 to 16.7 mM on ^{86}Rb FOR (upper panel), ^{45}Ca FOR (middle panel) and insulin release (lower panel) from islets perifused in the absence (○) and presence of BPDZ 44 (●; 10 μM) or diazoxide (■; 10 μM). Mean values (±SE) refer to 4–9 individual experiments.

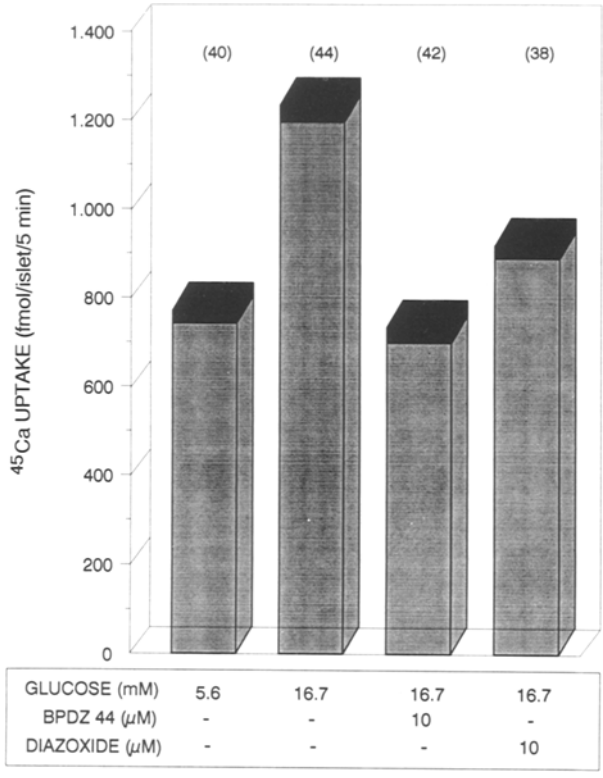

Figure 2. Effect of a rise in the glucose concentration from 5.6 to 16.7 mM on ^{45}Ca uptake by islets incubated in the absence and presence of BPDZ 44 (10 μM) or diazoxide (10 μM). Dark section at tops of bars corresponds to SEM. Figures in parentheses are numbers of individual experiments.

and sustained inhibition of ^{45}Ca outflow and insulin output (data not shown). The inhibitory effects of BPDZ 44 were always more pronounced than those of diazoxide ($p < 0.001$ in each case).

In the last set of experiments, ^{45}Ca uptake was measured over short term incubation periods (5 min) in order to estimate Ca^{2+} inflow[5]. A rise in the concentration of glucose from 5.6 to 16.7 mM significantly increased ^{45}Ca uptake ($p < 0.001$) (fig. 2). The presence of BPDZ 44 (10 μM) in the incubation medium completely abolished this glucose-stimulated short term ^{45}Ca uptake (fig. 2) whereas the presence of diazoxide (10 μM) only reduced it ($p < 0.001$). Thus, ^{45}Ca uptake averaged 741.8 ± 29.1 fmol/islet/5 min in the presence of 5.6 mM glucose; 1193.9 ± 39.1 fmol/islet/5 min in the presence of 16.7 mM glucose; 697.6 ± 35.2 fmol/islet/5 min in glucose-stimulated islets exposed to BPDZ 44 and 889.0 ± 29.6 fmol/islet/5 min in glucose-stimulated islets exposed to diazoxide.

Discussion

The present results demonstrate that at low concentrations BPDZ 44 completely abolished the release of insulin in response to a stepwise rise in extracelluar glucose concentration. Under the same experimental conditions, diazoxide only reduced by half the capacity of glucose to provoke insulin output. In islets exposed throughout to a medium containing an insulinotropic concentration of glucose (16.7 mM), the inhibitory effect of a low concentration of BPDZ 44 on the glucose-induced insulin release was again more marked than that of diazoxide. Thus, these data reveal that the pyridothiadiazine derivative BPDZ 44 is more active than the reference molecule diazoxide in inhibiting the insulin releasing process.

BPDZ 44 was also shown to be more active than diazoxide in affecting ^{45}Ca outflow from perifused rat pancreatic islets. Indeed, a low concentration of BPDZ 44 completely suppressed the increase in ^{45}Ca outflow mediated by a rise in the extracellular concentration of glucose whilst diazoxide failed to abolish but rather reduced this cationic response to glucose. Likewise, the inhibitory effect of BPDZ 44 on ^{45}Ca outflow from islets perifused throughout in the presence of 16.7 mM glucose was more pronounced than that of diazoxide. Under the two latter experimental conditions, the ^{45}Ca fractional outflow rate is known to reflect a sustained stimulation of isotopic exchange between influent ^{40}Ca and effluent ^{45}Ca (ref. 6). Thus, these findings suggest that BPDZ 44 is a more powerful inhibitor of Ca^{2+} entry into the islet cells than diazoxide. Such a proposal is further supported by the observation that BPDZ 44 but not diazoxide completely suppressed the stimulatory effect of glucose on short term ^{45}Ca uptake.

The effects of BPDZ 44 and diazoxide on Ca^{2+} inflow, however, are not mediated by a direct antagonistic action of these drugs on the B-cell Ca^{2+} channels. Earlier observations clearly indicated that their primary effects resulted from the activation of ATP-sensitive K^+ channels[1-4,7]. The K^+ channel activation is expected to hyperpolarize the B-cell membrane and will, in turn, restrict the opening of voltage-sensitive Ca^{2+} channels, decrease Ca^{2+} entry and ultimately inhibit the secretory process.

In this respect, it was interesting to notice that the increase in K^+ permeability provoked by BPDZ 44 was more marked than that evoked by diazoxide. Indeed, BPDZ 44 was much more potent than diazoxide in enhancing the basal ^{86}Rb outflow from islets perifused in the presence of 5.6 mM glucose. As a result, the effects of BPDZ 44 on both Ca^{2+} inflow and insulin output were more pronounced.

In conclusion, our data reveal that BPDZ 44 and diazoxide provoke similar modifications in ^{86}Rb outflow, ^{45}Ca outflow, ^{45}Ca uptake and insulin release from perfused and/or incubated rat pancreatic islets. However, the effects of a low concentration of BPDZ 44 on both the ionic and secretory events are clearly more marked than those of diazoxide. This difference implies that BPDZ 44 could be a valuable tool for characterizing further the structural requirements for ATP-sensitive K^+ channel activation and selectivity.

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* To whom correspondence should be addressed.

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