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K_{ATP} channel openers: Tissue selectivity of original 3-alkylaminopyrido- and 3-alkylaminobenzothiadiazine 1,1-dioxides

Philippe Lebrun [a,1,*], *Bénédicte Becker* [a,1], *Nicole Morel* [b], *Philippe Ghisdal* [b], *Marie-Hélène Antoine* [a], *Pascal de Tullio* [c], *Bernard Pirotte* [c]

[a] *Laboratory of Pharmacology, Faculty of Medicine (CP617), Université Libre de Bruxelles (ULB), Route de Lennik 808, B-1070 Brussels, Belgium*

[b] *Laboratory of Cellular Physiology, Faculty of Medicine, Université Catholique de Louvain, Brussels, Belgium*

[c] *Laboratory of Medicinal Chemistry, Drug Research Center, Université de Liège, Liège, Belgium*

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ABSTRACT

The present study was designed to further evaluate the biological effects and tissue selectivity of new 3-alkylaminobenzo- and 3-alkylaminopyridothiadiazine 1,1-dioxides bearing identical branched alkylamino chains at the 3-position. These original compounds were compared with their parent molecules; namely the K_{ATP} channel openers diazoxide and pinacidil.

All tested 3-alkylamino-substituted derivatives provoked a marked, concentration-dependent inhibition of the glucose-induced insulin secretion from rat pancreatic islets. The 3-alkylaminopyridothiadiazine 1,1-dioxides evoked a weak vasorelaxant activity whilst their 7-halo-substituted benzothiadiazine counterparts elicited pronounced, concentration-dependent, relaxations of rat aortic rings. The myorelaxant effect of the different drugs was tightly correlated with their capacity to increase ^{86}Rb outflow (^{42}K substitute) from prelabelled and perifused rat aortic rings. EC_{50}/IC_{50} ratios (vascular/pancreatic) revealed a pronounced selectivity of the 3-alkylaminopyridothiadiazine 1,1-dioxides towards the pancreatic endocrine tissue. Structure–activity relationships suggest that, besides the requirement of an electronegative pole at the 7-position of the heterocycle, a minimal steric hindrance confers an optimal insulin-secreting cell selectivity. Lastly, radioisotopic, electrophysiological and pharmacological investigations indicate that the marked vasorelaxant properties of the 3-alkylaminobenzothiadiazine 1,1-dioxides are related to the activation of smooth muscle K_{ATP} channels.

1. Introduction

Among the different types of K^+ channels so far described, the ATP-sensitive K^+ channels (K_{ATP} channels), which are mainly regulated by the cytosolic [ATP]/[ADP] ratio, couple the metabolic state of the cell to the cellular membrane potential [1].

K_{ATP} channels are octameric proteins composed of four inwardly rectifying potassium channel subunits (Kir 6.x) and four regulatory sulfonylurea receptor (SUR.x) subunits [1–3].

* *Corresponding author*. Tel.: +32 2 5556221; fax: +32 2 5556356.
E-mail address: plebrun@ulb.ac.be (P. Lebrun).

[1] Both authors contributed equally to the work.

Abbreviations: FOR, fractional outflow rate; K_{ATP}, ATP-sensitive K^+; GLIB, glibenclamide; AUC, area under the curve.

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Different variants of Kir 6.x (Kir 6.1, Kir 6.2) and SUR x (SUR 1, SUR 2A, SUR 2B) have been characterized and their multiple combinations lead to K_{ATP} channel subtypes [1–6]. These K_{ATP} channels have been identified in a wide range of cell types including insulin-secreting cells (Kir 6.2/SUR 1 subunits) [4] and vascular smooth muscle cells (Kir 6.1 or Kir 6.2/SUR 2B subunits) [5,6]. Moreover, they are involved in many cellular processes and have been shown to regulate the insulin-secreting response [4] and to control the vascular tone [5,6].

The therapeutic potential of K^+_{ATP} channel modulation is vast, including the treatment of arterial hypertension as well the treatment and/or prevention of metabolic disorders such as type I or type II diabetes, obesity and hyperinsulinaemia [7–12].

During the last decade [7–11] numerous pharmacological agents that can either act as blockers or openers of K_{ATP} channels have been developed.

K_{ATP} channel openers comprise different chemical classes such as benzothiadiazine dioxides, cyanoguanidines, benzopyranes, thioamides, pyridinic-derived nitric esters, pyrimidine N-oxide sulfates and tertiary carbinols [7–9,12,13]. In the last few years, we have developed original 3-alkylaminobenzothiadiazine 1,1-dioxides and 3-alkylaminopyridothiadiazine 1,1-dioxides [14–18]. The latter derivatives may be regarded as hybrid compounds between diazoxide and pinacidil; two well known K_{ATP} channel openers (Fig. 1). Most of these newly synthesized drugs have been shown to reduce the insulin secretory process and/or to exhibit vasorelaxant properties [14,15,17,18].

The main objective of the present study was to further characterize the tissue selectivity of some selected 3-alkylaminobenzo-and 3-alkylaminopyridothiadiazine 1,1-dioxides. We have compared, on a vascular and a rat pancreatic pharmacological model, the effects of several benzothiadiazine dioxides with those of pyridothiadiazine dioxides bearing identical branched alkylamino chains at the 3-position (Fig. 2). Additional investigations were also conducted to characterize the mechanism underlying the myorelaxant activity of the 3-alkylaminobenzothiadiazine 1,1-dioxides.

2. Materials and methods

All experiments were performed with aortae, mesenteric arteries or pancreatic islets isolated from fed Wistar rats. The laboratory animal care was approved by the local ethics committees of the Université Libre de Bruxelles and the Université Catholique de Louvain.

2.1. Measurement of insulin secretion from incubated rat pancreatic islets

Pancreatic islets were isolated by the collagenase method and freshly isolated islets were used for measurements of insulin secretion [16].

Groups of 10 islets, each derived from the same batch of islets, were pre-incubated for 30 min at 37 °C in 1 ml of a physiological salt medium (in mM: NaCl 115, KCl 5, $CaCl_2$ 2.5, $MgCl_2$ 1, $NaHCO_3$ 24) supplemented with 2.8 mM glucose, 0.5% (w/v) dialyzed albumin and equilibrated against a mixture of O_2 (95 %) and CO_2 (5%). The islets were then incubated at 37 °C for a further 90 min in 1 ml of the same medium containing 16.7 mM glucose and, in addition, either the pyridothiadiazine or the benzothiadiazine derivative. The release of insulin was measured radioimmunologically using rat insulin as a standard [16]. Each dose–action curve was repeated on different islets populations and the n values refer to the number of individual experiments.

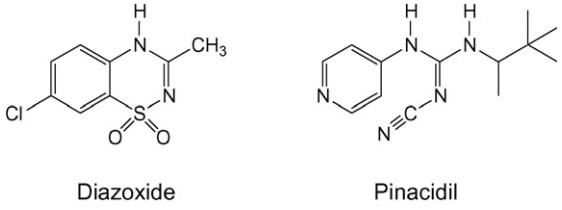

Fig. 1 – Chemical structure of diazoxide and pinacidil.

2.2. Measurements of ^{86}Rb outflow from perifused rat aorta

The medium used for incubating, washing or perfusing the aortic rings was a bicarbonate-buffered solution (in mM: NaCl 115, KCl 5, $CaCl_2$ 2.5, $MgCl_2$ 1, $NaHCO_3$ 24, glucose 5)

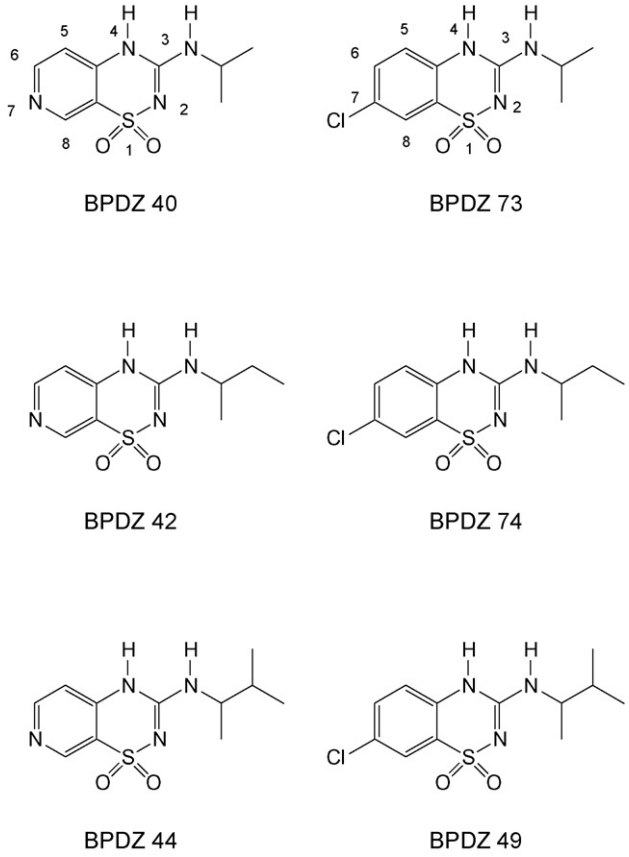

Fig. 2 – Chemical structure of original 3-alkylaminopyridothiadiazine 1,1-dioxides (left) and 3-alkylaminobenzothiadiazine 1,1-dioxides (right).

equilibrated against a mixture of O_2 (95%) and CO_2 (5 %). Segments (≈2 mm long) of rat aorta were first incubated for 30 min at 37 °C. The segments were then incubated for a further 60 min in the same medium to which ^{86}Rb (0.15–0.25 mM, 50 μCi/ml) had been added. After incubation, the segments were washed 4 times with a non-radioactive medium in order to remove extracellular radioactivity. Groups of 2 aortic rings were then placed in a perifusion chamber and the perifusate was delivered at a constant flow rate (1.0 ml/min). The outflow of ^{86}Rb (a tracer for K^+) from perifused aortic rings was measured as described previously [19,20]. The efflux of ^{86}Rb (cpm/min) was expressed as a fractional outflow rate (FOR: % of instantaneous aorta content per min). Measurements of ^{86}Rb outflow from perifused rat aortic rings were conducted in the presence of 30 mM extracellular KCl in order to mimic the experimental conditions used to measure muscle tension.

2.3. Measurements of tension in rat aorta

The thoracic aorta was removed, cut into transverse rings (3–4 mm), and adhering fat and connective tissue was detached [21]. After removal of the endothelium, the segments were suspended under 2 g load in an organ bath containing Krebs bicarbonate-buffered solution (in mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 5). The solution maintained at 37 °C was continuously oxygenated with a mixture of 95% O_2 and 5% CO_2. After equilibration for 60 min, isometric contractions were measured with a force–displacement transducer. Contractile activity was induced by increasing the extracellular concentration of K^+ (30 or 80 mM KCl). When a plateau of tension was reached, drugs were added to the preparation cumulatively to a maximum concentration of 300 μM. Some experiments were repeated in the continuous presence of 1 or 10 μM glibenclamide.

2.4. Measurements of membrane potential in rat mesenteric arteries

Smooth muscle cell membrane potential was measured as described previously [22]. A segment of the rat superior mesenteric artery, ±2 mm in length, was inverted and mounted in a myograph. The mesenteric segment was continuously perfused with a physiological solution (in mM: NaCl 122, KCl 5.9, $NaHCO_3$ 15, $MgCl_2$ 1.25, $CaCl_2$ 1.25 and glucose 10) gassed with 95% O_2-5% CO_2 and maintained at 37 °C. Vessels were maintained under zero force for 60 min. A passive diameter–tension curve was constructed and the vessel was set at a tension equivalent to that generated at 0.9 times the diameter of the vessel at 100 mmHg.

Measurement of the smooth muscle membrane potential was made with a glass microelectrode (type GC 120F-15, Clark Electromedical Instruments, U.K.) filled with 1.5 M KCl and advanced through the luminal surface of the arterial segment. The input resistance of the microelectrodes varied between 50 and 80 MΩ. Potential differences were measured with reference (reference electrode from Clark Electromedical Instrument, type E208) to the grounded bath by means of a Dagan amplifier (U.S.A.). Criteria for a successful impalement were an abrupt drop in voltage on entry of the microelectrode into the cell, stable membrane potential for at least 2 minutes, and a sharp return to zero on withdrawal of the electrode.

2.5. Drugs

The media contained, as required, albumin (fraction V, Sigma–Aldrich, Belgium), diazoxide (Sigma–Aldrich), pinacidil (Sigma–Aldrich), glibenclamide (MP Biomedicals, Germany), 3-alkylaminopyridothiadiazine 1,1-dioxides and/or 3-alkylaminobenzothiadiazine 1,1-dioxides (synthesized at the Laboratory of Medicinal Chemistry, Université de Liège, Belgium). Pyridothiadiazine derivatives included BPDZ 40: 3-isopropylamino-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide; BPDZ 42: 3-(l-methylpropyl)amino-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide and BPDZ 44: 3-(1,2-dimethylpropyl) amino-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide. Benzothiadiazine derivatives included BPDZ 49: 7-chloro-3-(l,2-dimethylpropyl)amino-4*H*-1,2,4-benzothiadiazine 1, 1-dioxide; BPDZ 73: 7-chloro-3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide and BPDZ 74: 7-chloro-3-(l-methylpropyl)amino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide. Diazoxide, pinacidil, glibenclamide, pyridothiadiazine and benzothiadiazine derivatives were dissolved in dimethylsulfoxide which was added to both control and test media at final concentrations not exceeding 0.1% (v/v).

When high concentrations of extracellular K^+ (>30 mM) were used, the concentration of extracellular NaCl was lowered to keep osmolarity constant.

2.6. Calculations

Results are expressed as the mean (±S.E. mean). The area under the curve (AUC) for the ^{86}Rb fractional outflow rate was estimated in each individual experiment from the integrated outflow of ^{86}Rb observed during stimulation (45th–68th min) after correction for basal value (40th–44th min). The IC_{50} value for insulin release (concentration giving a 50% reduction of the secretory response to 16.7 mM glucose) was assessed using Excell software. For the rat aortic rings, EC_{50} values (drug concentrations inducing half-maximum inhibition of the plateau phase induced by KCl) were assessed from concentration–response curves using Datanalyst software (EMKA Technologies, France). The statistical significance of the differences between mean data was assessed by use of paired or unpaired Student's *t*-test.

3. Results

3.1. Effects of 3-alkylaminopyridothiadiazine 1,1-dioxides on insulin release from rat pancreatic islets, contractile activity and ^{86}Rb outflow from rat aortic rings

The ability of the 3-alkylaminopyridothiadiazine 1,1-dioxides to inhibit insulin release was evaluated on isolated rat pancreatic islets incubated in the presence of 16.7 mM extracellular glucose.

All tested 3-alkylamino compounds provoked a concentration-dependent inhibition of the glucose-induced insulin secretion. On looking at the calculated IC_{50} values (Table 1),

Table 1 – Effects of 3-alkylaminopyridothiadiazine 1,1-dioxides, diazoxide and pinacidil, on insulin release from rat pancreatic islets, contractile activity and ^{86}Rb outflow from rat aortic rings

Compound	IC_{50} (μM) pancreatic islets	EC_{50} (μM) aortic rings	^{86}Rb-AUC (%/min) aortic rings
BPDZ 40	23.0 ± 6.3(3)	>300.0 (8)	0.00 ± 0.01 (5)
BPDZ 42	9.6 ± 3.3(5)	>300.0 (9)	0.00 ± 0.01 (3)
BPDZ 44	2.7 ± 0.3(6)	141.1 ± 11.2(9)	0.14 ± 0.02(4)
Diazoxide	18.4 ± 2.2(5)	22.4 ± 1.9(15)	0.34 ± 0.02(14)
Pinacidil	202.3 ± 28.3(3)	0.62 ± 0.17(15)	1.71 ± 0.13(6)

IC_{50}, EC_{50} and ^{86}Rb-AUC values are expressed as mean ± S.E. mean with the number of individual experiments in parenthesis. IC_{50} is the drug concentration (μM) eliciting 50% reduction in 16.7 mM glucose-induced insulin release whilst EC_{50} is the drug concentration (μM) eliciting 50% relaxation of the 30 mM KCl-induced contraction. ^{86}Rb-AUC: area under the curve for ^{86}Rb outflow observed during stimulation (compounds tested at 100 μM).

the rank order of potency for these 3-alkylaminopyridothiadiazine 1,1-dioxides was: BPDZ 44 > BPDZ 42 > BPDZ 40. Pinacidil, used as a reference compound, behaved as a weak inhibitor of the insulin releasing process (Table 1).

The biological effect of the compounds was further evaluated on the contractile activity of 30 mM KCl-depolarized rat aorta rings (Table 1).

The 3-alkylaminopyridothiadiazine 1,1-dioxides were found to express, if any, a weak myorelaxant activity. The data further indicate that the different compounds were far less potent than pinacidil and diazoxide at reducing the vascular tone (Table 1).

Measurements of ^{86}Rb (^{42}K substitute) outflow from prelabelled and perifused rat aortic rings also revealed that the addition of 100 μM BPDZ 40 or 100 μM BPDZ 42 to the perifusing medium did not affect the rate of ^{86}Rb outflow (data not shown and Table 1). BPDZ 44 (100 μM) provoked a slight increase whilst pinacidil (100 μM) and diazoxide (100 μM) elicited a sustained rise in ^{86}Rb outflow from perifused rat aortic rings (data not shown and Table 1).

Comparison of the EC_{50} and IC_{50} values (Table 1), as well as calculation of the EC_{50}/IC_{50} ratio, allows to assess the apparent tissue selectivity (vascular versus pancreatic) of the different compounds.

As expected, pinacidil exhibited a clear-cut affinity for the vascular tissue (EC_{50}/IC_{50} ratio = 0.003). Diazoxide, which has been reported to lack tissue selectivity, was found to be nearly equipotent on the pancreatic and the vascular tissue (EC_{50}/IC_{50} = 1.22).

By contrast, the 3-alkylaminopyridothiadiazine 1,1-dioxides, which failed to exert any marked effect on the vascular smooth muscle whilst being potent inhibitors of the insulin releasing process, expressed a pronounced selectivity for the pancreatic tissue. The EC_{50}/IC_{50} ratio amounted to 52 for BPDZ 44, was above 31 for BPDZ 42 and above 13 for BPDZ 40.

3.2. *Effects of 3-alkylaminobenzothiadiazine dioxides on insulin release from rat pancreatic islets, contractile activity and ^{86}Rb outflow from rat aortic rings*

The 3-alkylaminobenzothiadiazine 1,1-dioxides inhibited, in a concentration-dependent manner, insulin output from glucose-stimulated rat pancreatic islets (Table 2).

According to the IC_{50} values, the rank order of potency was: BPDZ 73 > BPDZ 74 > BPDZ 49.

Diazoxide, the parent molecule, was less potent than the 3-alkylamino derivatives at inhibiting the insulin secretory rate (Table 2).

In contrast to the previously described pyridothiadiazine dioxides bearing identical alkylamino chains at the 3-position, these 3-alkylaminobenzothiadiazine 1,1-dioxides derivatives can be regarded as potent myorelaxant agents. Indeed, in rat aortic rings exposed to 30 mM extracellular K^+, the cumulative application (10^{-7} to 3.10^{-4} M) of BPDZ 73, BPDZ 74 and BPDZ 49 elicited concentration-dependent relaxations (Table 2). The 3-alkylaminobenzothiadiazine derivatives were less potent than pinacidil but, apart from BPDZ 73, evoked a more pronounced vasorelaxant effect than diazoxide.

Table 2 further reveals a tight correlation (r = 0.91) between the myorelaxant effects of the 3-alkylaminobenzothiadiazine 1,1-dioxides (BPDZ 73, BPDZ 74 and BPDZ 49) and their ability to provoke an increment in the rate of ^{86} Rb outflow (all

Table 2 – Effects of 3-alkylaminobenzothiadiazine 1,1-dioxides, diazoxide and pinacidil, on insulin release from rat pancreatic islets, contractile activity and ^{86}Rb outflow from rat aortic rings

Compound	IC_{50} (μM) pancreatic islets	EC_{50} (μM) aortic rings	^{86}Rb-AUC (%/min) aortic rings
BPDZ 73	0.55 ± 0.10(3)	34.9 ± 3.0(11)	0.23 ± 0.03(4)
BPDZ 74	3.0 ± 0.2(4)	10.1 ± 2.4(10)	0.46 ± 0.04(8)
BPDZ 49	14.5 ± 1.2(5)	1.14 ± 0.20(10)	0.87 ± 0.09(9)
Diazoxide	18.4 ± 2.2(5)	22.4 ± 1.9(15)	0.34 ± 0.02(14)
Pinacidil	202.3 ± 28.3(3)	0.62 ± 0.17(15)	1.71 ± 0.13(6)

IC_{50}, EC_{50} and ^{86}Rb-AUC values are expressed as mean ± S.E. mean with the number of individual experiments in parenthesis. IC_{50} is the drug concentration (μM) eliciting 50% reduction in 16.7 mM glucose-induced insulin release whilst EC_{50} is the drug concentration (μM) eliciting 50% relaxation of the 30 mM KCl-induced contraction. ^{86}Rb-AUC: area under the curve for ^{86}Rb outflow observed during stimulation (compounds tested at 100 μM).

compounds tested at 100 μM) from prelabelled rat aortic rings. BPDZ 49 was the most potent at reducing the vascular tone and also at increasing ^{86}Rb outflow.

The magnitude of the enhancing effect of diazoxide (100 μM) and pinacidil (100 μM) on the ^{86}Rb outflow rate was also linked to the vasorelaxant properties of these two reference compounds (Table 2).

3.3. *Effects of 3-alkylaminobenzothiadiazine 1,1-dioxides on the contractile activity of 30 and 80 mM K$^+$-depolarized rat aortic rings incubated in the absence or presence of glibenclamide*

Glibenclamide, a hypoglycaemic sulfonylurea known to close the ATP-sensitive K^+ channels [23–25], reduced the myorelaxant effects of the 3-alkylaminobenzothiadiazine 1,1-dioxides (Table 3).

The presence of glibenclamide in the bathing medium provoked a concentration-dependent rightward shift of the dose–response curve for BPDZ 73, BPDZ 74 and BPDZ 49. Thus, when calculating the EC_{50} ratio for experiments conducted in the absence and presence of 10 μM glibenclamide, the hypoglycaemic sulfonylurea displaced the concentration–response curve 3.2 fold for BPDZ 73, 3.0 fold for BPDZ 74 and 42.2 fold for BPDZ 49.

Under the same experimental conditions, glibenclamide provoked a 8.4 displacement of the concentration–response curve to the reference compound diazoxide (Table 3).

The presence of glibenclamide in the physiological medium did not affect the baseline tension or the contractile responses to KCl (30 mM).

The vasorelaxant activity of the 3-alkylaminobenzothiadiazine 1,1-dioxides was further examined on rat aortic rings precontracted by high concentrations of extracellular K^+ (80 mM).

Table 3 indicates that, under the latter experimental condition, the myorelaxant effects of BPDZ 73, BPDZ 74 and BPDZ 49 were markedly weakened. The vasorelaxant activity of diazoxide was also reduced in 80 mM K^+-depolarized rat aortic rings (Table 3).

3.4. *Effects of 3-alkylaminobenzothiadiazine 1,1-dioxides on ^{86}Rb outflow from rat aortic rings perifused in the presence or absence of glibenclamide*

Fig. 3 clearly shows that the addition of BPDZ 49 (100 μM) provoked a rapid, marked and sustained increase in ^{86}Rb outflow from prelabelled and perifused rat aortic rings.

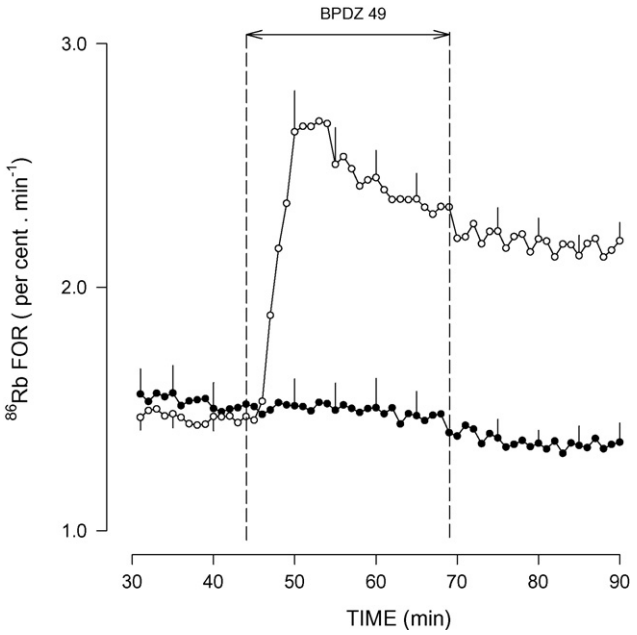

Fig. 3 – Effect of BPDZ 49 (100 μM) on ^{86}Rb outflow from rat aortic rings perifused throughout in the absence (○) or presence of glibenclamide (●; 10 μM). Mean values (±S.E. mean) refer to 6–9 individual experiments.

When the same experiment was repeated in the presence of glibenclamide (10 μM) in the physiological medium, the basal (min 31–44) rate of ^{86}Rb outflow was unaffected. The stimulatory effect of BPDZ 49 was, however, completely abolished (Fig. 3).

Additional experiments further indicated that glibenclamide (10 μM) inhibited the rise in ^{86}Rb outflow induced by BPDZ 73 (100 μM) and BPDZ 74 (100 μM) (data not shown).

Incidentally, diazoxide (100 μM) and pinacidil (100 μM) also provoked a glibenclamide (10 μM)-inhibitable increase in ^{86}Rb outflow (data not shown).

3.5. *Effects of BPDZ 49 on membrane potential in rat mesenteric arteries*

Mean value of resting membrane potential of smooth muscle cells of the rat mesenteric artery was -44.2 ± 0.8 mV ($n = 4$).

Addition of BPDZ 49 to the perfusion solution shifted the membrane potential to more negative values (Fig. 4, upper

Table 3 – Myorelaxant effects (EC_{50}) of 3-alkylaminobenzothiadiazine 1,1-dioxides and diazoxide on 30 and 80 mM K$^+$-induced contractions of rat aortic rings incubated in the absence or presence of glibenclamide

Compound	30 mM KCl	30 mM KCl + 1 μM GLIB	30 mM KCl + 10 μM GLIB	80 mM KCl
BPDZ 73	41.5 ± 2.6 (13)	117.1 ± 11.4(11)	132.0 ± 9.4 (10)	110.9 ± 7.6 (10)
BPDZ 74	16.9 ± 2.0 (7)	44.3 ± 1.8(11)	50.3 ± 3.0 (12)	61.2 ± 4.1 (8)
BPDZ 49	1.1 ± 0.3 (6)	42.9 ± 2.0 (10)	46.4 ± 1.7(8)	40.1 ± 3.1 (8)
Diazoxide[a]	19.5 ± 2.7(6)	85.8 ± 22.2 (6)	163.4 ± 41.2 (6)	>300 (6)

EC_{50} is the drug concentration (μM) eliciting 50% relaxation of the 30 or 80 mM KCl-induced contraction. Number in parentheses refers to the number of individual experiments performed in each group. GLIB: glibenclamide.

[a] Pirotte et al. [18].

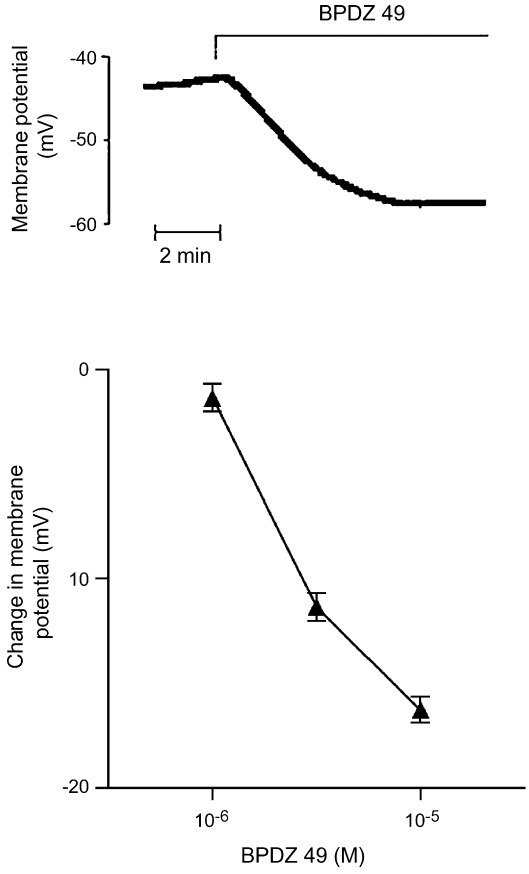

Fig. 4 – Effect of BPDZ 49 on the membrane potential of rat mesenteric artery smooth muscle cells. *Upper panel*: Typical experimental trace showing the effect of BPDZ 49 (10 μM) on membrane potential. *Lower panel*: Concentration–effect curve for the effect of BPDZ 49 on the membrane potential of mesenteric artery smooth muscle cells. Mean values (±S.E. mean) refer to 3–4 determinations.

panel) and the amplitude of the hyperpolarizing effect of BPDZ 49 was clearly concentration-dependent (Fig. 4, lower panel). In the presence of 10 μM BPDZ 49, membrane potential averaged -60.5 ± 1.2 mV ($n = 4$).

By comparison, under identical experimental conditions, the K^+ channel opener cromakalim (10 μM) hyperpolarized mesenteric artery smooth muscle cells from -42 ± 1.7 mV to -68 ± 2 mV ($n = 3$) [22].

4. Discussion

The present experimental data further document the ability of original 3-alkylaminobenzo- and 3-alkylaminopyridothiadiazine 1,1-dioxides to reduce the glucose-induced insulin output from incubated rat pancreatic islets.

The different drugs tested in this study, bearing short alkylamino side chain at the 3-position, exhibited a biological activity equivalent or more pronounced than that of the reference compounds. Moreover, the nature of the hydrocarbon chain on the exocyclic nitrogen atom at the 3 position modulated the inhibitory effect of the compounds on the insulin releasing process. In the 3-alkylaminopyridothiadiazine 1,1-dioxide series, the rank order of potency was dimethylpropyl > methylpropyl > isopropyl. By contrast, the rank order of potency was isopropyl > methylpropyl > dimethylpropyl for the 3-alkylaminobenzothiadiazine 1,1-dioxide series.

Previous investigations conducted both with 3-alkylaminobenzo- and 3-alkylaminopyridothiadiazine 1,1-dioxides indicated that their ability to inhibit the insulin secretory rate resulted from their capacity to activate plasma membrane ATP-sensitive K^+ channels [16,26–31].

In a second series of experiments, the effects of the 3 alkylaminopyrido- and 3-alkylaminobenzothiadiazine 1,1-dioxides, putative smooth muscle K_{ATP} channel openers, were characterized on the mechanical activity of vascular smooth muscle.

The 3-alkylaminopyridothiadiazine 1,1-dioxides, up to 300 μM, barely affected the contractile activity of 30 mM KCl-depolarized rat aorta rings. Such compounds, which are structurally related to diazoxide and pinacidil, did not express the myorelaxant properties of their parent molecules.

By contrast, 3-alkylaminobenzothiadiazine 1,1-dioxides, bearing an identical alkylamino side chain at position 3, provoked marked vasorelaxant effects. The myorelaxant capacity of the compounds was again subjected to the size and branching of the alkylamino side chain. Drugs bearing methylpropylamino and dimethylpropylamino chains at position 3 were even more potent than diazoxide at relaxing vascular smooth muscle. Thus, the presence of a hydrophobic group at position 7 together with an increase in the steric hindrance appears to amplify the vasorelaxant properties of these original compounds.

Preliminary studies indicated that, for 3-alkylamino-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides, the inhibitory effect on the insulin releasing process was amplified with the increase of the size and the branching of the alkyl chain introduced on the exocyclic nitrogen atom at the 3-position, until reaching a maximum active size corresponding to the alkyl chain of BPDZ 44 (1,2-dimethylpropyl). A further increase in the size and steric hindrance of this alkyl chain resulted in a reduction of the biological activity on insulin-secreting cells [15]. Concerning the 7-chloro-3-alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides, recent investigations suggested that a marked inhibitory effect on the insulin releasing process could only be observed with compounds bearing a very short alkyl or cycloalkyl side chain on the exocyclic nitrogen atom at the 3-position (i.e. ethyl, isopropyl, cyclobutyl). An increase in the size of this alkyl chain rapidly resulted in a loss of activity on insulin-secreting cells and an improvement of the myorelaxant properties of the compounds [14].

The present study, conducted with the two series of compounds simultaneously tested under identical experimental conditions, strongly highlights the critical importance of both the steric impact of the 3-alkylamino side chain and that resulting from the nature of the 7-position in the two heterocyclic systems. Thus, 3-alkylamino-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides, bearing a nitrogen atom at the 7-position instead of the more bulky halo-substituted carbon atom of 7-chloro-3-alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides, seem to accommodate more easily bulky

branched alkylamino chains at the 3-position. Such a feature could be of significant importance for the interaction of the compounds with their target sites on pancreatic insulin-secreting cells (formally the SUR 1 subunit of the pancreatic K_{ATP} channels). By contrast, a wider hydrophobic pocket located on the SUR 2B subunit of the vascular smooth muscle K_{ATP} channels could probably allow to accommodate more bulky groups. In the latter case, an increase in the size and the branching of the alkylamino chain should improve the hydrophobic interactions between the compounds and their receptors. This proposal appears to be correct both for the benzo- and the pyridothiadiazine dioxides since the most active pyridothiadiazine dioxide on the vascular smooth muscle (i.e. BPDZ 44) was indeed the compound bearing the more bulky alkylamino chain.

Incidentally, a thienothiadiazine dioxide targeting the insulin-secreting cell (NN414) and closely related to the pyrido- and benzothiadiazine dioxides reported in the present work was recently developed by Novo Nordisk. This K_{ATP} channel opener was selected for clinical trials on the basis of its selectivity of action for the pancreatic versus the smooth muscle tissue [12,32]. Regarding the thienothiadiazine dioxides, it was also observed that the nature of the alkylamino chain at the 3-position affected the tissue selectivity [33,34]. Although the impact of length and ramification of the latter alkylamino chain was not firmly established (in contrast to our present observations), the best compromise for activity and selectivity towards the insulin-secreting cells was displayed by compound NN414 bearing a short branched 1-methylcyclopropylamino chain at the 3-position and a chlorine atom at the 6-position [33,34].

Thus, and taking into account all these findings, it seems reasonable to speculate that the different spatial requirements of both the SUR 1 and SUR 2B binding sites influence the functional responses. Such an hypothesis has already been proposed for other pharmacological compounds, i.e. the binding of nonsteroidal antiinflammatory drugs on the catalytic site of the cyclooxygenase isoforms COX-1 and COX-2. Indeed, the COX-2 binding site accommodates more bulky structures than the COX-1 site; a feature which led to the design of original COX-2 selective inhibitors [35].

Regarding the weak vasorelaxant properties of pyridothiadiazine dioxides and the subsequent selectivity indexes (EC_{50}/IC_{50} ratio), the data suggest that this series of heterocyclic compounds can be expected to provide more examples of pancreatic B-cell selective drugs than the benzothiadiazine dioxides series (BPDZ 73 must be considered as an exception in this series). Such findings support the view that, although an electronegative pole is required at the 7-position (the nitrogen atom of the pyridine ring or a chlorine atom linked to the benzene ring) of the heterocycles to warrant B-cell selectivity, a minimal steric hindrance confers an optimal tissue selectivity.

The present results further suggest that the vasorelaxant properties of the 3-alkylaminobenzothiadiazine 1,1-dioxides are related to the activation of smooth muscle ATP-sensitive K^+ channels.

Firstly, the drugs increased ^{86}Rb (^{42}K substitute) outflow from prelabelled and perifused rat aorta rings. Although the measurement of ^{86}Rb outflow probably underestimates the real changes in K^+ fluxes [20,36], such findings indicate that the 3-alkylaminobenzothiadiazine 1,1-dioxides provoked a rise in the membrane permeability to K^+. Secondly, BPDZ 49, a representative compound of this chemical class, provoked a concentration-dependent hyperpolarization of the artery smooth muscle cells. Such a hyperpolarizing effect can also be attributed to an increase in membrane K^+ permeability. Thirdly, the vasorelaxant properties of the 3-alkylaminobenzothiadiazine 1,1-dioxides were correlated to their capacity to increase ^{86}Rb outflow from prelabelled aortic rings. Fourthly, the increase in ^{86}Rb outflow induced by the 3-alkylaminobenzothiadiazine 1,1-dioxides was inhibited by glibenclamide, a K_{ATP} channel blocker [21,23–25]. Fifthly, in 30 mM K^+-depolarized rat aorta rings, the presence of glibenclamide in the bathing medium provoked a concentration-dependent rightward shift of the dose–response curve for 3-alkylaminobenzothiadiazine 1,1-dioxides. Sixthly, and as reported previously for numerous K_{ATP} channel openers [20,21,37], the myorelaxant effect of the different compounds was reduced in aortic rings exposed to high extracellular K^+ concentrations (80 mM). Lastly, the mechanical, pharmacological, radioisotopic and electrophysiological responses to 3-alkylaminobenzothiadiazine 1,1-dioxides are reminiscent of those exhibited by recognized K_{ATP} channel openers [19–21,25,37].

All together, these findings suggest that the 3-alkylaminobenzothiadiazine 1,1-dioxides activate K_{ATP} channels in vascular smooth muscle and that such a process results in myorelaxant effects.

In summary, the present data point out to an apparent pancreatic endocrine tissue selectivity of 3-alkylaminopyridothiadiazine 1,1-dioxides bearing, at position 3, short alkylamino side chains. Moreover, the 7-halobenzenic counterparts of such pyridothiadiazine dioxides express less tissue selectivity but exhibit marked vasorelaxant properties attributable to the activation of smooth muscle ATP-sensitive K^+ channels.

Structure–activity relationships and apparent tissue selectivities deduced from the present study will help to identify and guide the design of original drugs targeting ATP sensitive K^+ channels in insulin-secreting cells. Such compounds could be expected to be valuable drugs in therapeutical applications such as the prevention and/or treatment of diabetes, hyperinsulinaemia and obesity.

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