ELSEVIER

Biochemical Pharmacology 62 (2001) 923–928

Biochemical Pharmacology

Effects of tolbutamide and *N*-benzoyl-D-phenylalanine (NBDP) on the regulation of $[Ca^{2+}]_i$ oscillations in mouse pancreatic islets

Sigurd Lenzen*, Thomas Peckmann

Institute of Clinical Biochemistry, Hannover Medical School, D-30623 Hannover, Germany

Received 14 March 2001; accepted 18 May 2001

Abstract

The sulfonylurea derivative, tolbutamide, and the phenylalanine derivative, *N*-benzoyl-D-phenylalanine (NBDP), both of which stimulate insulin secretion through interaction with the sulfonylurea receptor (SUR1), were studied for their ability to increase the $[Ca^{2+}]_i$ and to interact with the glucose-induced slow large amplitude $[Ca^{2+}]_i$ oscillations in isolated mouse pancreatic islets. Tolbutamide as well as NBDP induced $[Ca^{2+}]_i$ oscillations of extremely slow frequency. Both compounds also lowered the threshold for the glucose-induced slow large amplitude $[Ca^{2+}]_i$ oscillations and significantly reduced their frequency in intact islets as well as in single pancreatic beta cells. These $[Ca^{2+}]_i$ oscillations apparently require a glucokinase-mediated glycolytic flux. This conclusion is supported by the observations that KIC, a mitochondrial fuel, cannot replace glucose in this synergism and that mannoheptulose, an inhibitor of glucokinase and glucose-induced insulin secretion, abolishes these slow $[Ca^{2+}]_i$ oscillations. In conclusion, these compounds potentiate the effect of glucose. This additive effect is the likely result of a synergistic closing action upon the ATP-sensitive K^+ (K_{ATP}) channel, mediated in the case of glucose through an action upon the channel protein itself of ATP generated in glucose catabolism and in the case of tolbutamide and NBDP upon the sulfonylurea receptor (SUR1) associated with this channel.

Keywords: Pancreatic islets; Cytoplasmic Ca^{2+}; Slow $[Ca^{2+}]_i$ oscillations; Tolbutamide; *N*-Benzoyl-D-phenylalanine (NBDP)

1. Introduction

The sulfonylurea derivative, tolbutamide, and the phenylalanine derivative, *N*-benzoyl-D-phenylalanine (NBDP), belong to groups of hypoglycaemic agents that induce insulin secretion through interaction with the sulfonylurea receptor (SUR1) in the plasma membrane of the pancreatic beta cell [1–3]. In the present investigation, we studied these compounds for their ability to increase the $[Ca^{2+}]_i$ in isolated pancreatic mouse islets.

Glucose, the principal physiological stimulus of insulin secretion, induces slow large amplitude $[Ca^{2+}]_i$ oscillations [4–7] in pancreatic islets and their frequency decreases with the increase in the glucose concentration [8]. These slow glucose-induced $[Ca^{2+}]_i$ oscillations in pancreatic beta cells are apparently dependent upon an interaction of glycolytically generated ATP with ATP-sensitive K^+ (K_{ATP}) channels.

We therefore also investigated in the present study the ability of the two agents to interact with the generation of the typical glucose-induced slow large amplitude $[Ca^{2+}]_i$ oscillations [4–7] in order to obtain information upon their interplay with glucose when interacting with the ATP-sensitive K^+ (K_{ATP}) channel in the beta cell membrane.

The results show that both compounds exhibit a synergistic additive closing action together with glucose upon the ATP-sensitive K^+ (K_{ATP}) channel and thereby lower the threshold for the glucose-induced slow large amplitude $[Ca^{2+}]_i$ oscillations and reduce their frequency.

2. Materials and methods

2.1. Solutions and chemicals

N-Benzoyl-D-phenylalanine (NBDP), α-ketoisocaproic acid (KIC), D-mannoheptulose, HEPES, and collagen were purchased from Sigma. Tolbutamide was from Aventis. Fura-2 acetoxymethyl ester was from Molecular Probes, collagenase (type P) from Roche Diagnostics, and bovine

* Corresponding author.

Abbreviations: KIC, α-ketoisocaproic acid; NBDP, *N*-benzoyl-D-phenylalanine; and SUR1, sulfonylurea receptor.

0006-2952/01/$ – see front matter
PII: S0006-2952(01)00719-5

serum albumin (fraction V) from Miles. Tissue culture media and supplements were from GIBCO Life Technologies. All other chemicals of analytical grade were obtained from Merck.

2.2. Isolation, preparation, tissue culture, and perifusion of pancreatic islets and of single islet cells

Pancreatic islets were isolated from 6- to 10-week-old female NMRI mice by collagenase digestion in Krebs–Ringer bicarbonate medium [9] supplemented with 20 mM HEPES, 5 mM glucose, and 0.1% albumin at pH 7.4 and 37°. Single islet cells were prepared from islets by treatment with Ca^{2+}-deficient Krebs–Ringer medium containing 0.5 mM EGTA for 10 min at room temperature followed by vortexing for 1–2 min. Cells were dispersed in RPMI-1640 medium. Twenty-five microlitres of cell suspension containing 2000–3000 cells was put on a cover slip and allowed to attach for 2 hr at 37°. Thereafter, 3 mL RPMI-1640 medium was added. Single islets and islet cells on collagen-coated cover slips were kept before the experiment in RPMI-1640 tissue culture medium containing 5 mM glucose and supplemented with 10% (v/v) fetal bovine serum (FBS), 100 U/mL of penicillin, and 100 μg/mL of streptomycin for 2 to 7 days (islets) and 1–4 days (cells), respectively [8].

2.3. Measurement of intracellular free Ca^{2+} concentration

Single islets or islet cells attached to cover slips were loaded with fura-2 acetoxymethyl ester by incubation in Krebs–Ringer medium containing 25 mM HEPES and supplemented with 5 mM glucose, 3% albumin, and 3 μM fura-2 acetoxymethyl ester for 45 (islets) or 30 min (cells) at 37°. In the experiments, pancreatic islets and islet cells were perifused with the same Krebs–Ringer medium containing 1 mM $CaCl_2$, but without albumin. Before switching to the test medium for 60 min, tissue was perifused for 5 min with basal medium containing 5 mM glucose. Only in the experiments where the effects of other test compounds were studied in the absence of glucose, the basal preperifusion medium contained also no glucose. Cover slips with fura-2-loaded islets or cells were used as the exchangeable bottom of a custom-made perifusion chamber with a volume of 300 μL. Perifusion of islets or cells was performed through two cannulas for inflow and outflow with a flow rate of 1 mL/min using a peristaltic pump (Ismatec ISM 820). Tissue was perifused for 5 min with basal medium. After changing the perifusion medium, there was a lag time of about 4 sec before the new solution reached the tissue. Recordings shown in the figures were corrected for this delay. Chamber and cannulas were heated to maintain a medium temperature of 37°. The chamber was mounted onto the stage of an inverted microscope (Zeiss Axiovert 135TV). The microscope was equipped for epifluorescence fluorometry with a 400-nm dichroic mirror (Till Photonics) and a 40 X FLUAR® objective (Zeiss). Excitation light was generated by a monochromator type B (Till Photonics). Intensity of monochromatic light was reduced to 5% by neutral density filters (Laser Components) [10]. $[Ca^{2+}]_i$ was recorded by dual wavelength excitation at 340 and 380 nm, and emission at 510 nm was measured by a photonmultiplier (SMT) digitised by an ITC-16 analogue digital converter (islets) (Instrutech Co.) or a slow scan CCD camera (single cells) (Theta Systems). Emission at 510 nm caused by the fluorescence excitation at 340 and 380 nm as well as the ratio of emission caused by the excitations (340 nm/380 nm) were recorded continuously by the computer PULSE software, version 8.07 (HEKA-Elektronik) with a frequency of 1 Hz (islets) or the FUCAL software, version 5.12 (TILL Photonics) with a frequency of 0.33 Hz (single cells). Background fluorescence of the cells was deducted from the values. Calibration of the system was performed as described [11] using the formula of Grynkiewicz *et al*. [12] for calculation of the Ca^{2+} concentrations.

2.4. Statistical analyses

Data are expressed as mean values ± SEM and tested for statistical significance with Student's *t*-test.

3. Results

Tolbutamide (0.2 mM) as well as NBDP (0.2 mM) induced in the absence of glucose very slow oscillations of $[Ca^{2+}]_i$ in isolated mouse pancreatic islets (Fig. 1 and Table 1). In the presence of a substimulatory glucose concentration (5 mM), which alone is an insufficient concentration to induce oscillations, tolbutamide (0.2 mM) as well as NBDP (0.2 mM) induced slow large amplitude $[Ca^{2+}]_i$ oscillations with a pattern characteristic for 20 mM glucose (Fig. 1 and Table 1). The mean increase of the $[Ca^{2+}]_i$ in the presence of tolbutamide (0.2 mM) or NBDP (0.2 mM) at 5 mM glucose was as high already as at 10 mM glucose in the absence of these compounds (Table 1).

Tolbutamide (0.2 mM) as well as NBDP (0.2 mM) slowed down the frequency of the glucose-induced slow large amplitude $[Ca^{2+}]_i$ oscillations at glucose concentrations of 10, 15, and 20 mM (Table 1). This means that the significant decrease in the frequency of the slow $[Ca^{2+}]_i$ oscillations which is observed when the glucose concentration is increased from a lower level to 20 mM (Table 1) is apparent at 10 mM already in the presence of tolbutamide (0.2 mM) or NBDP (0.2 mM) (Table 1). At glucose concentrations of 15 and 20 mM, tolbutamide (0.2 mM) and NBDP (0.2 mM) even further reduced the frequency (Table 1). The mean increase in the $[Ca^{2+}]_i$ was not affected by the addition of tolbutamide (0.2 mM) or NBDP (0.2 mM) at glucose concentrations between 10 and 20 mM (Table 1).

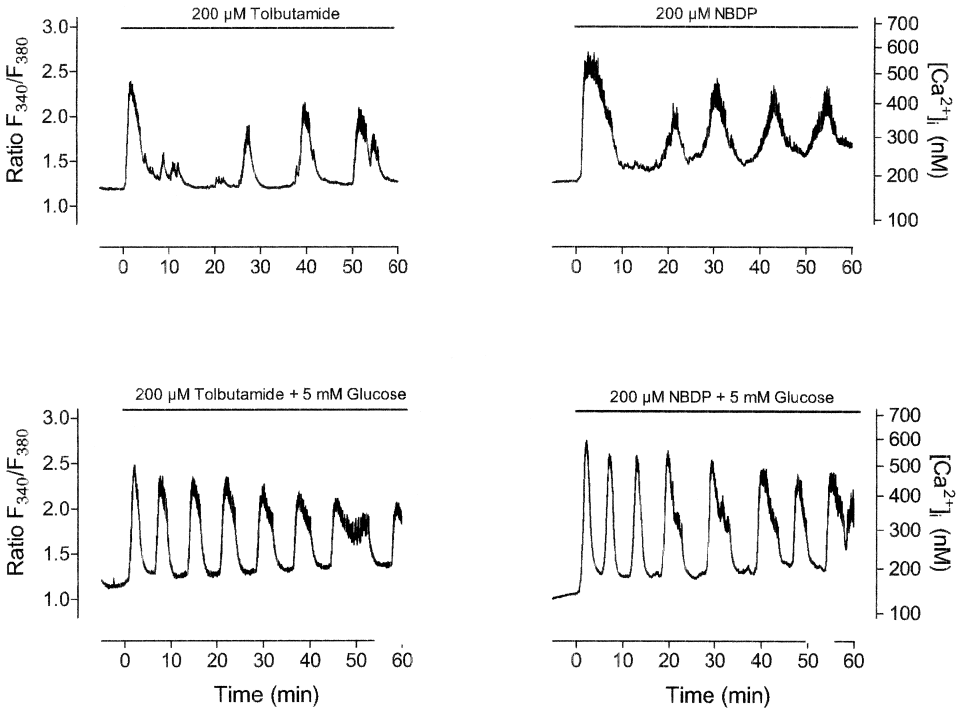

Fig. 1. Effect of tolbutamide (0.2 mM) or NBDP (0.2 mM) in the absence or presence of glucose (5 mM) on $[Ca^{2+}]_i$ in isolated mouse pancreatic islets. Basal medium was switched at time 0 to test medium containing tolbutamide (0.2 mM) or NBDP (0.2 mM) alone or supplemented additionally with glucose (5 mM) for 60 min as indicated by the bars. Depicted are typical recordings from 4 or 5 experiments, each.

When the concentration of tolbutamide was increased to 0.8 mM, which is a concentration of this sulfonylurea compound with an *in vitro* secretory potency equivalent to 15 mM glucose with respect to the two compounds to induce an immediate insulin secretory response of the pancreatic beta cells [13,14], the same biphasic immediate response of the $[Ca^{2+}]_i$ was observed (Fig. 2). The first peak increase of $[Ca^{2+}]_i$ which appears within the first min after switching from the basal to the medium with the test compounds is induced by tolbutamide (0.8 mM). After a transient return to near basal levels, the second $[Ca^{2+}]_i$ increase 2–3 min after the beginning of the stimulation is induced by glucose (15 mM) (Fig. 2). NBDP (0.8 mM) in the combination with glucose (15 mM) induced a biphasic response comparable to that of tolbutamide (Fig. 2).

Mannoheptulose (2 mM), an inhibitor of glucokinase and glucose-induced insulin secretion, reversibly suppressed the appearance of the glucose-induced (20 mM) slow $[Ca^{2+}]_i$

Table 1
Comparison of the frequencies of slow oscillations of $[Ca^{2+}]_i$ induced by tolbutamide or NBDP in isolated mouse pancreatic islets in dependence upon the glucose concentration compared with those of glucose alone

Glucose concentration	Tolbutamide concentration	NBDP concentration	Frequency (min^{-1})	Mean increase of $[Ca^{2+}]_i$ (nM)	No of Exps. (N)
10 mM			0.42 ± 0.03	151 ± 11	11
15 mM			0.42 ± 0.03	180 ± 11	6
20 mM			0.21 ± 0.03*	184 ± 18	4
0 mM	0.2 mM		0.09 ± 0.01	59 ± 9	5
5 mM	0.2 mM		0.17 ± 0.03	149 ± 18	5
10 mM	0.2 mM		0.22 ± 0.02**	148 ± 7	6
15 mM	0.2 mM		0.14 ± 0.02**	226 ± 50	4
20 mM	0.2 mM		0.14 ± 0.03**	226 ± 20	4
0 mM		0.2 mM	0.09 ± 0.01	85 ± 11	5
5 mM		0.2 mM	0.15 ± 0.04	164 ± 26	5
10 mM		0.2 mM	0.16 ± 0.04**	178 ± 36	4
15 mM		0.2 mM	0.18 ± 0.03**	185 ± 33	5
20 mM		0.2 mM	0.14 ± 0.02**	200 ± 11	6

Depicted are mean values ± SEM and the numbers of experiments.
* $P < 0.05$ compared with 10 mM glucose alone.
** $P < 0.05$ compared with the corresponding glucose concentration in the absence of tolbutamide or NBDP, respectively.

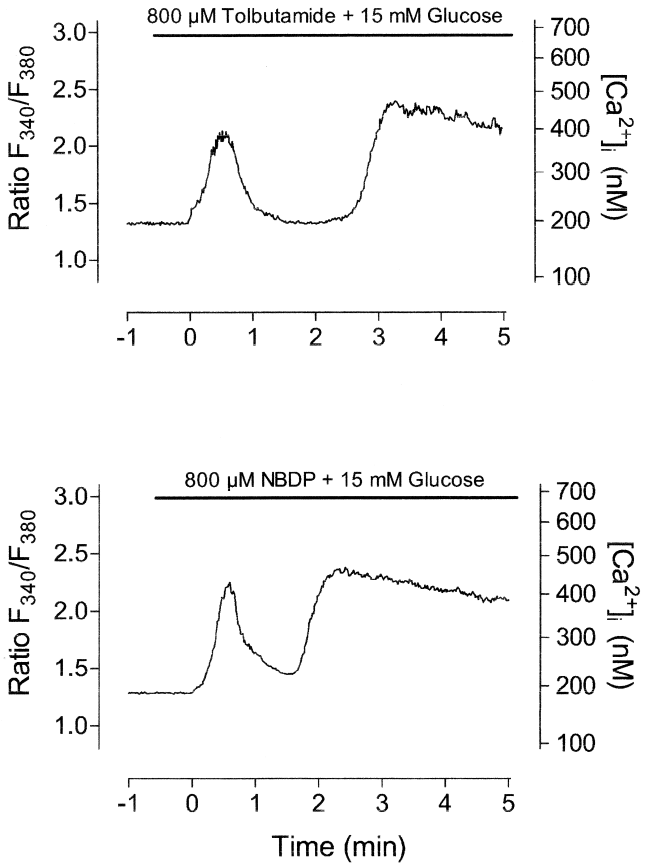

Fig. 2. Biphasic response of $[Ca^{2+}]_i$ in isolated mouse pancreatic islets in response to tolbutamide (0.8 mM) plus glucose (15 mM) or NBDP (0.8 mM) plus glucose (15 mM). Basal medium without test compound was switched at time 0 to test medium containing tolbutamide (0.8 mM) plus glucose (15 mM) or NBDP (0.8 mM) plus glucose (15 mM) for 5 min as indicated by the bars. Depicted are typical recordings from 4 experiments, each.

oscillations also in the presence of tolbutamide (0.2 mM) (Fig. 3).

Tolbutamide (0.2 mM), in the presence of KIC, a fuel of mitochondrial metabolic fate, both at a substimulatory concentration of 1 mM (Fig. 4) and at a stimulatory concentration of 5 mM (graph not shown), induced a permanent increase in the $[Ca^{2+}]_i$ but did not induce the characteristic slow $[Ca^{2+}]_i$ oscillations typical for glucose (Fig. 1).

Tolbutamide (0.2 mM), in the presence of 1 mM glucose, induced the same pattern of $[Ca^{2+}]_i$ increase without slow oscillations (graph not shown) as in the presence of 1 mM KIC (Fig. 4).

In analogy to the experiments with isolated pancreatic islets, tolbutamide (0.2 mM) as well as NBDP (0.2 mM), in the presence of 10 mM glucose, also significantly ($P < 0.05$) reduced the frequency of the glucose-induced $[Ca^{2+}]_i$ oscillations in isolated mouse pancreatic beta cells from 0.15 ± 0.01 (N = 16) at 10 mM glucose alone to 0.08 ± 0.01 (N = 4) and 0.08 ± 0.01 (N = 4), respectively (Fig. 5). This effect of the two compounds is comparable to that of a significant reduction ($P < 0.05$) of the frequency from

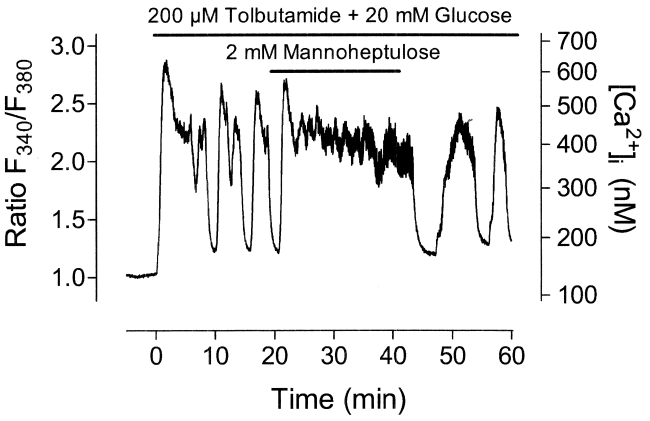

Fig. 3. Inhibitory effect of mannoheptulose (2 mM) on oscillations of $[Ca^{2+}]_i$ in isolated mouse pancreatic islets induced by tolbutamide (0.2 mM) plus glucose (20 mM). Basal medium was switched at time 0 to test medium (0.2 mM tolbutamide plus 20 mM glucose) for 60 min as indicated by the bar. From min 21–40, perifusion medium was supplemented additionally with mannoheptulose (2 mM). Depicted is a typical recording from 5 experiments.

0.15 ± 0.01 (N = 16) to 0.08 ± 0.01 (N = 4) which can be observed with isolated mouse pancreatic beta cells when the glucose concentration was increased from 10 to 20 mM [8].

4. Discussion

Glucose induces typical slow $[Ca^{2+}]_i$ oscillations in pancreatic beta cells [4–7]. Their frequency decreases with increasing glucose concentrations [8]. Tolbutamide as well as *N*-benzoyl-D-phenylalanine (NBDP) induced $[Ca^{2+}]_i$ oscillations of extremely slow frequency in pancreatic beta cells, significantly slower even than those induced by glucose. Such extremely slow oscillations have been reported before for tolbutamide [15] and an NBDP derivative [1]. Both compounds were also able to significantly reduce the

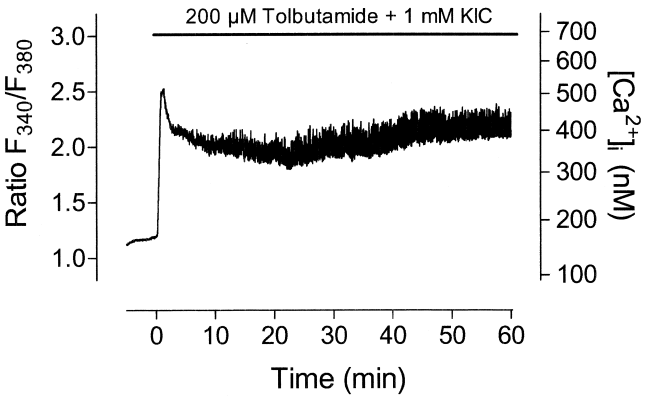

Fig. 4. Effect of KIC (1 mM) on $[Ca^{2+}]_i$ increased by tolbutamide (0.2 mM) in isolated mouse pancreatic islets. Basal medium was switched at time 0 to test medium containing tolbutamide (0.2 mM) plus KIC (1 mM) for 60 min as indicated by the bars. Depicted are typical recordings from 4 experiments, each.

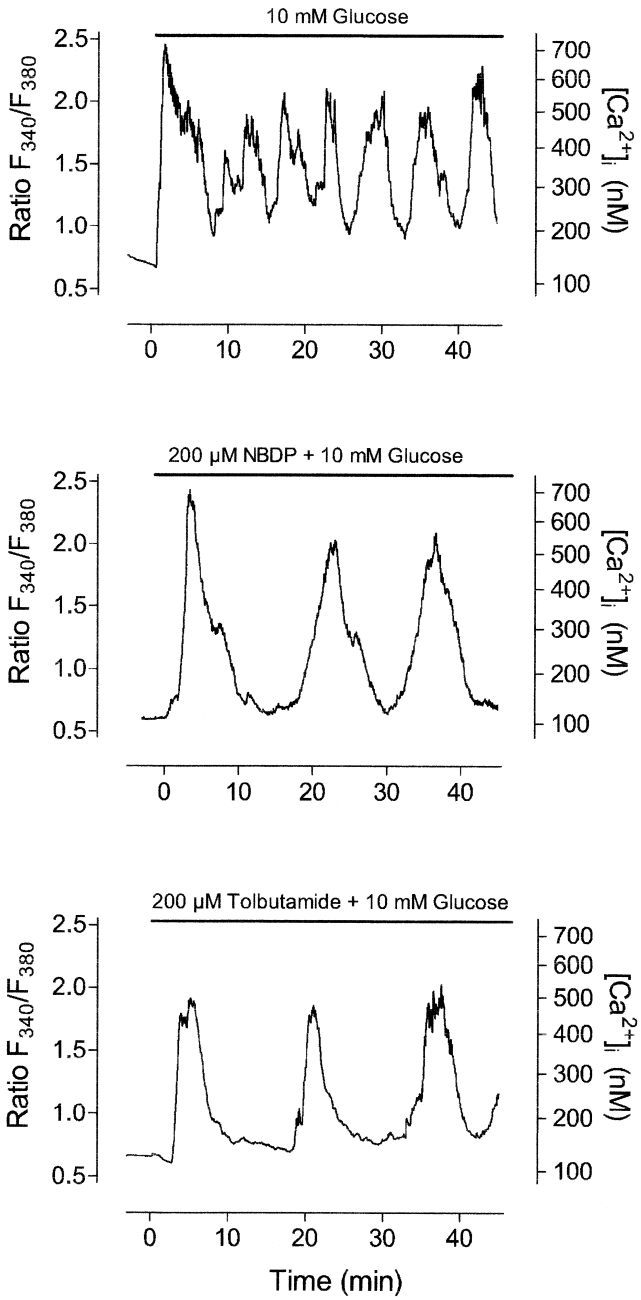

Fig. 5. Effect of tolbutamide (0.2 mM) or NBDP (0.2 mM) in the presence of glucose (10 mM) on $[Ca^{2+}]_i$ in isolated single mouse pancreatic beta cells compared with the effect of glucose (10 mM) alone. Basal medium was switched at time 0 to test medium for 45 min as indicated by the bars. Depicted are typical recordings from 4 experiments, each.

frequency of the glucose-induced slow $[Ca^{2+}]_i$ oscillations in intact islets as well as in single pancreatic beta cells. Thus, these compounds potentiate the effects of glucose. With respect to their effects upon insulin secretion, this synergism with glucose is also a well-known phenomenon [16,17].

The observation that mannoheptulose, an inhibitor of glucokinase and glucose-induced insulin secretion [18], can abolish the glucose-induced slow $[Ca^{2+}]_i$ oscillations in the presence of tolbutamide and NBDP indicates that these oscillations apparently require a glucokinase-mediated glycolytic flux. This conclusion is supported by the observations that in the presence of a low glucose concentration of 1 mM, which is insufficient for a glucokinase-mediated glycolytic flux as well as in the presence of the mitochondrial fuel KIC tolbutamide and NBDP were unable to produce slow $[Ca^{2+}]_i$ oscillations of a type characteristic for glucose in the physiological millimolar concentration range. Thus, the presence of glucose at concentrations high enough to support a glucokinase-mediated metabolic flux is apparently a prerequisite for tolbutamide and NBDP to reduce the frequency of glucose-induced slow $[Ca^{2+}]_i$ oscillations.

ATP, through interaction with ATP-sensitive K^+ (K_{ATP}) channels, depolarises the plasma membrane. But ATP also inhibits phosphofructokinase and through this mechanism it reduces the metabolic flux through glycolysis. This in turn reduces ATP generation which through relief of phosphofructokinase inhibition via fructose-1,6-bisphosphate-mediated phosphofructokinase activation, increases the glycolytic flux rate again. This is the metabolic basis for the generation of oscillations of metabolic flux through glycolysis [19]. Through interaction of ATP with the K_{ATP} channels in the plasma membrane, the resulting Ca^{2+} influx through voltage-sensitive Ca^{2+} channels is responsible for the generation of glucose-induced slow $[Ca^{2+}]_i$ oscillations in pancreatic beta cells [4,5,8].

The reason for the slowing down of the frequency of the glucose-induced slow $[Ca^{2+}]_i$ oscillations by tolbutamide and NBDP may be explained as follows. At the K_{ATP} channel tolbutamide and NBDP, through binding to the sulfonylurea receptor (SUR1) in the plasma membrane of the pancreatic beta cell, act in a synergistic manner with glucose, because both tolbutamide and NBDP, like glucose, increase Ca^{2+} uptake into the beta cell through voltage-sensitive Ca^{2+} channels [3,20]. This additive effect is possible because, unlike ATP [20] and other agents [21], tolbutamide and NBDP primarily interact with the sulfonylurea receptor (SUR1).

Further evidence for this interaction at the sulfonylurea receptor (SUR1) and the pore-forming subunit of the K_{ATP} channel (KIR6.2) protein complex is provided by the experiments in which a combination of a high concentration of tolbutamide or NBDP and a stimulatory concentration of glucose induced a biphasic immediate response of the $[Ca^{2+}]_i$ (Fig. 2). The first peak increase of $[Ca^{2+}]_i$ is induced by tolbutamide or NBDP, respectively [14], through interaction with the SUR1, the second by glucose [14] through ATP interacting with the KIR6.2. This experiment reproduces with respect to the Ca^{2+} response of the beta cell an analogous insulin secretion experiment performed many years ago in the perfused pancreas [13,14].

On the other hand, compounds such as tolbutamide increase energy consumption through stimulation of insulin secretion without supplying energy [16,17]. This reduces ATP and brings the $[Ca^{2+}]_i$ back to a basal level. As it takes

longer for glucose to replenish the depleted ATP stores in the presence of these compounds, it also takes longer for the phosphofructokinase inhibition through ATP to become effective again. In contrast to the K_{ATP} channel, tolbutamide and NBDP do not work in synergism with ATP at the phosphofructokinase.

Thus, the present investigation shows that tolbutamide and NBDP on the one hand and glucose on the other, in spite of their distinct mechanisms of interaction with the K_{ATP} channel protein complex, act synergistically. Through this closing action upon the K_{ATP} channel, sulfonylurea drugs and related compounds lower the threshold for glucose-induced slow $[Ca^{2+}]_i$ oscillations and mimic the physiological pattern of slow glucose-induced $[Ca^{2+}]_i$ oscillations in pancreatic beta cells. This ability of blood glucose-lowering sulfonylurea drugs and related agents [22] many be advantageous when these compounds are used in the therapy of defective glucose-induced insulin secretion in type 2 diabetes.

Acknowledgments

The skilful technical assistance of A. Petzold is gratefully acknowledged. Some of the results were obtained during thesis work by T.P.

References

[1] Fujitani S, Yada T. A novel D-phenylalanine-derivative hypoglycemic agent A-4166 increases cytosolic free Ca^{2+} in rat pancreatic beta-cells by stimulating Ca^{2+} influx. Endocrinology 1994;134:1395–400.

[2] Fujitani S, Ikenoue T, Akiyoshi M, Maki T, Yada T. Somatostatin and insulin secretion due to common mechanisms by a new hypoglycemic agent, A-4166, in perfused rat pancreas. Metabolism 1996;45:184–9.

[3] Panten U, Schwanstecher M, Schwanstecher C. Sulfonylurea receptors and mechanism of sulfonylurea action. Exp Clin Endocrinol Diabetes 1996;104:1–9.

[4] Hellman B, Gylfe E, Grapengiesser E, Lund PE, Berts A. Cytoplasmic Ca^{2+} oscillations in pancreatic beta-cells. Biochim Biophys Acta 1992;1113:295–305.

[5] Gylfe E, Grapengiesser E, Liu YJ, Dryselius S, Tengholm A, Eberhardson M. Generation of glucose-dependent slow oscillations of cytoplasmic Ca^{2+} in individual pancreatic beta cells. Diabetes Metab 1998;24:25–9.

[6] Henquin JC, Jonas JC, Gilon P. Functional significance of Ca^{2+} oscillations in pancreatic beta cells. Diabetes Metab 1998;24:30–6.

[7] Soria B, Martin F. Cytosolic calcium oscillations and insulin release in pancreatic islets of Langerhans. Diabetes Metab 1998;24:37–40.

[8] Lenzen S, Lerch M, Peckmann T, Tiedge M. Differential regulation of $[Ca^{2+}]_i$ oscillations in mouse pancreatic islets in response to glucose, α-ketoisocaproic acid, glyceraldehyde and glycolytic intermediates. Biochim Biophys Acta 2000;1523:65–72.

[9] Lenzen S. Insulin secretion by isolated perfused rat and mouse pancreas. Am J Physiol 1979;236:E391–400.

[10] Grapengiesser E. Cell photodamage, a potential hazard when measuring cytoplasmic Ca^{2+} with fura-2. Cell Struct Funct 1993;18:13–7.

[11] Diederichs F. A decrease of both $(Ca^{2+})_e$ and $(H^+)_e$ produces cell damage in the perfused rat heart. Cell Calcium 1997;22:487–96.

[12] Grynkiewicz G, Poenie M, Tsien RY. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. J Biol Chem 1985;260:3440–50.

[13] Lenzen S. The immediate insulin secretory response of the isolated perfused rat pancreas to tolbutamide and glucose. FEBS Lett 1975;49:407–8.

[14] Lenzen S. The immediate insulin secretory response of the rat pancreas to glucose compared with tolbutamide and other secretagogues. Diabetes 1978;27:27–34.

[15] Grapengiesser E, Gylfe E, Hellman B. Sulfonylurea mimics the effect of glucose in inducing large amplitude oscillations of cytoplasmic Ca^{2+} in pancreatic beta-cells. Mol Pharmacol 1990;37:461–7.

[16] Panten U, Zünkler BJ, Scheit S, Kirchhoff K, Lenzen S. Regulation of energy metabolism in pancreatic islets by glucose and tolbutamide. Diabetologia 1986;29:648–54.

[17] Panten U, Burgfeld J, Goerke F, Rennicke M, Schwanstecher M, Wallasch A, Zünkler BJ, Lenzen S. Control of insulin secretion by sulfonylureas, meglitinide and diazoxide in relation to their binding to the sulfonylurea receptor in pancreatic islets. Biochem Pharmacol 1989;38:1217–29.

[18] Lenzen S, Panten U. Signal recognition by pancreatic B-cells. Biochem Pharmacol 1988;37:371–8.

[19] Tornheim K. Are metabolic oscillations responsible for normal oscillatory insulin secretion? Diabetes 1997;46:1375–80.

[20] Ashcroft FM, Gribble FM. ATP-sensitive K^+ channels and insulin secretion: their role in health and disease. Diabetologia 1999;42:903–19.

[21] Monks LK, Cosgrove KE, Dunne MJ, Ramsden CA, Morgan NG, Chan SL. Affinity isolation of imidazoline binding proteins from rat brain using 5-amino-efaroxan as a ligand. FEES Lett 1999;447:61–4.

[22] Bailey CJ. Hypoglycaemic and anti-hyperglycaemic drugs for the control of diabetes. Proc Nutr Soc 1991;50:619–30.