Biochemical Pharmacology, Vol. 45, No. 9, pp. 1845–1849, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

MODULATION OF THE INSULINOTROPIC ACTION OF GLIBENCLAMIDE AND GLIMEPIRIDE BY NUTRIENT SECRETAGOGUES IN PANCREATIC ISLETS FROM NORMOGLYCEMIC AND HYPERGLYCEMIC RATS

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(*Received* 17 *November* 1992; *accepted* 25 *January* 1993)

Abstract—In perifused pancreatic islets from euglycemic rats, the secretory response to either glibenclamide or glimepiride (1.0 μM each) increases as a function of the concentration of D-glucose (2.8–16.7 mM) present in the perifusion medium. On the contrary, the sulfonylurea-induced increment in ^{45}Ca efflux from prelabeled islets decreases at increasing concentrations of the hexose. Neither glibenclamide nor glimepiride affect D-glucose metabolism in isolated islets, as judged from the production of ^{3}HOH from D-[5-^{3}H]glucose or the generation of $^{14}CO_2$, as well as ^{14}C-labeled amino acids and acidic metabolites, from D-[3,4-^{14}C]glucose, D-[2-^{14}C]glucose and D-[6-^{14}C]glucose. The insulinotropic action of the hypoglycemic sulfonylureas is not impaired in islets prepared from rats infused for 48 hr with a hypertonic solution of D-glucose. The dimethyl ester of succinic acid is more efficient than D-glucose in supporting the insulin-releasing effect of glibenclamide or glimepiride. Thus, although the insulinotropic action of hypoglycemic sulfonylureas appears unaffected in a model of B-cell glucotoxicity, a potentiation of their secretory effects might be expected, in non-insulin-dependent diabetes, from the combined administration of succinic acid methyl ester.

It has long been known that the magnitude and even the time course of the pancreatic B-cell secretory response to hypoglycemic sulfonylureas are modulated by the ambient concentration of D-glucose [1–3]. The major and interrelated aims of the present study were to explore whether such a glucose dependency displays a comparable pattern with glibenclamide and a new highly potent hypoglycemic sulfonylurea, namely glimepiride [4], whether it involves interference of these hypoglycemic agents with the metabolism of D-glucose in the islet cells, whether the secretory response to these agents is modified in a model of B-cell glucotoxicity, and whether succinic acid dimethyl ester (SAD), which was recently introduced as an efficient non-glucidic nutrient secretagogue [5–7], is also able, like D-glucose, to support the insulinotropic action of glibenclamide and glimepiride.

MATERIALS AND METHODS

All experiments were conducted in islets isolated from fed albino rats by the collagenase method [8].

The methods used to measure insulin release from perifused [9] or incubated [8] islets, to monitor ^{45}Ca efflux from prelabeled islets [9] and to measure the generation of $^{14}CO_2$, ^{14}C-labeled amino acids and acidic metabolites and ^{3}HOH by islets exposed to both D-[^{14}C]glucose and D-[5-^{3}H]glucose [10, 11] were reported previously in the cited references.

In one series of experiments, pancreatic islets were isolated from rats (234 ± 2 g body weight; N = 3) infused for 48 hr from 9 a.m. onwards with a hypertonic solution of D-glucose (1.67 M) administered at a rate close to 4.6 mmol of D-glucose per hour [12]. In these animals, the glycemia, measured by teststrips (Medi-Test, Macherey-Nagel; Düren, F.R.G.) in blood samples collected from the tail of the rats, increased from an initial value of 7.5 ± 0.2 mM (N = 3) to 20.4 ± 1.5 mM (N = 9) during the first day and 17.4 ± 1.3 mM (N = 9) during the second day, each of these mean values being derived from measurements made at approximately 12 a.m., 4 p.m. and 8 a.m. on the next day. The rats were disconnected from the infusion pump at 9 a.m. and immediately killed by decapitation. At that time, their plasma insulin concentration averaged 448.5 ± 80.5 μU/mL. The isolation of islets from these animals by the collagenase method was conducted in media containing 16.7 mM D-glucose, i.e. a concentration close to that found *in vivo*.

All results, including those mentioned above, are presented as the mean value (±SEM) together with the number of individual determinations (N). The statistical significance of differences between mean values was assessed by use of Student's *t*-test.

RESULTS

Glucose-dependency of the cationic and secretory responses to glibenclamide and glimepiride in perifused islets

The effect of glibenclamide and glimepiride (1.0 μM each) upon ^{45}Ca efflux and insulin release from prelabeled islets was examined in a perifusion system at concentrations of D-glucose ranging from 2.8 to 16.7 mM. The results concerning the

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Table 1. Effects of glibenclamide and glimepiride upon ^{45}Ca efflux and insulin release from islets perifused at increasing concentrations of D-glucose

D-Glucose (mM)	Glibenclamide (1.0 μM)	Glimepiride (1.0 μM)
Increment in ^{45}Ca fractional outflow rate (10^{-3}/min)		
2.8	7.46 ± 0.42 (6)	6.60 ± 0.35 (6)
5.6	6.02 ± 0.38 (4)	6.80 ± 0.73 (5)
8.3	4.35 ± 0.31 (6)	3.95 ± 0.25 (5)
16.7	2.17 ± 0.25 (4)	1.62 ± 0.11 (5)
Increment in insulin release (nU/islet per min)		
2.8	47 ± 10 (6)	43 ± 10 (7)
5.6	306 ± 120 (4)	172 ± 33 (5)
8.3	730 ± 146 (6)	803 ± 133 (5)
16.7	743 ± 44 (4)	649 ± 38 (5)

sulfonylurea-induced increment in ^{45}Ca fractional outflow rate and insulin output are summarized in Table 1. Figure 1 illustrates the data recorded in the presence of either 5.6 or 16.7 mM D-glucose. Those obtained in the presence of either 2.8 or 8.3 mM D-glucose were already illustrated in a prior publication [13]. In these experiments, no significant difference was observed between the results obtained with glibenclamide and glimepiride. The drug-induced increment in ^{45}Ca fractional outflow rate between the 45th and 68th min of perifusion averaged, after correction for the mean paired reading recorded from the 40th to 44th min inclusive, 6.78 ± 0.25 10^{-3}/min (N = 21) in the presence of 2.8–5.6 mM D-glucose, 4.17 ± 0.21 10^{-3}/min (N = 11) in the presence of 8.3 mM D-glucose and 1.86 ± 0.16 10^{-3}/min (N = 9) in the presence of 16.7 mM D-glucose. This progressive decrease of the sulfonylurea-induced increment in ^{45}Ca fractional outflow rate at increasing concentrations of D-glucose contrasted with a progressive increase of the sulfonylurea-induced increment in insulin output. When computed in the same manner as described for ^{45}Ca outflow, the increment in insulin output attributable to glibenclamide or glimepiride indeed averaged 45 ± 7 nU/islet per min (N = 13) in the presence of 2.8 mM D-glucose, 232 ± 57 nU/islet per min (N = 9) in the presence of 5.6 mM D-glucose and 731 ± 54 nU/islet per min (N = 20) in the presence of 8.3 and 16.7 mM D-glucose.

As shown in Fig. 1, the stimulation of insulin release by either glibenclamide or glimepiride in the presence of either 5.6 or 16.7 mM D-glucose represented an immediate and sustained phenomenon, persisting for at least 20 min after removal of these agents from the perifusion medium. A comparable situation was previously documented in the presence of either 2.8 or 8.3 mM D-glucose [13].

Effect of glibenclamide and glimepiride upon D-glucose metabolism

In the presence of 6.0 mM D-glucose, neither glibenclamide nor glimepiride, when tested at a concentration of 1.0 μM, affected significantly the conversion of D-[5-^{3}H]glucose to ^{3}HOH and that of either D-[3,4-^{14}C]glucose, D-[2-^{14}C]glucose or D-[6-^{14}C]glucose to $^{14}CO_2$ and ^{14}C-labeled amino acids and acidic metabolites (Table 2). Whether in the absence or presence of each hypoglycemic sulfonylurea, the oxidation of D-[3,4-^{14}C]glucose exceeded that of D-[2-^{14}C]glucose, which was itself more efficiently oxidized than D-[6-^{14}C]glucose (Fig. 2). The differences in oxidation rate were matched by complementary differences in the yield of ^{14}C-labeled amino acids and acidic metabolites.

Effect of glibenclamide upon insulin release in a model of B-cell glucotoxicity

The next series of experiments were performed with islets prepared from rats infused for 48 hr with a hypertonic solution of D-glucose.

The release of insulin by islets prepared from the glucose-infused rats averaged 3.05 ± 0.22 μU/min per islet (N = 12) after 10 min of perifusion in the presence of 16.7 mM D-glucose. During the first 45 min of perifusion, conducted in the sole presence of the hexose, the output of insulin slowly declined (Fig. 3). Nevertheless, even at the 44th min, the output of insulin remained higher ($P < 0.025$) in the islets from glucose-infused rats (2.59 ± 0.33 μU/min per islet; N = 12) than in those from control animals (1.58 ± 0.14 μU/min per islet; N = 9). The administration of either glibenclamide or glimepiride (1.0 μM) provoked, within 120 sec, a sizeable and sustained increment of insulin output. However, when the administration of the sulfonylureas was interrupted at the 70th min, no further obvious change was observed in the slowly descending pattern of insulin output.

Modulation of the insulinotropic action of glibenclamide and glimepiride by succinic acid dimethyl ester

In the last series of experiments, we have compared the effects of D-glucose and SAD upon the secretory response to either glibenclamide or glimepiride. As shown in Table 3, in the absence of the latter agents, the release of insulin was slightly, but not significantly, higher in the presence of 6.0 mM D-glucose than 10.0 mM SAD, both secretory rates exceeding ($P < 0.025$ or less) basal insulin output. In the absence of an exogenous nutrient, glibenclamide and glimepiride only caused a modest, but significant ($P < 0.02$), increase in insulin output. The sulfonylurea-induced increment in insulin output was much higher in the presence of either D-glucose or SAD. In contrast to the data recorded in the absence of sulfonylurea, the secretory rate recorded in the presence of either glibenclamide or glimepiride was higher ($P < 0.001$) in islets exposed to 10.0 mM SAD than 6.0 mM D-glucose.

DISCUSSION

The present data confirm that the secretory response to glibenclamide is more marked in the presence than absence of D-glucose [2]. The glucose-dependency of the B-cell secretory response displayed a comparable pattern in the case of glibenclamide and glimepiride, the drug-induced increment in insulin output reaching its maximal value in the range of hexose concentrations between 8.3 and 16.7 mM. This contrasted with a progressive decrease in the sulfonylurea-induced increment

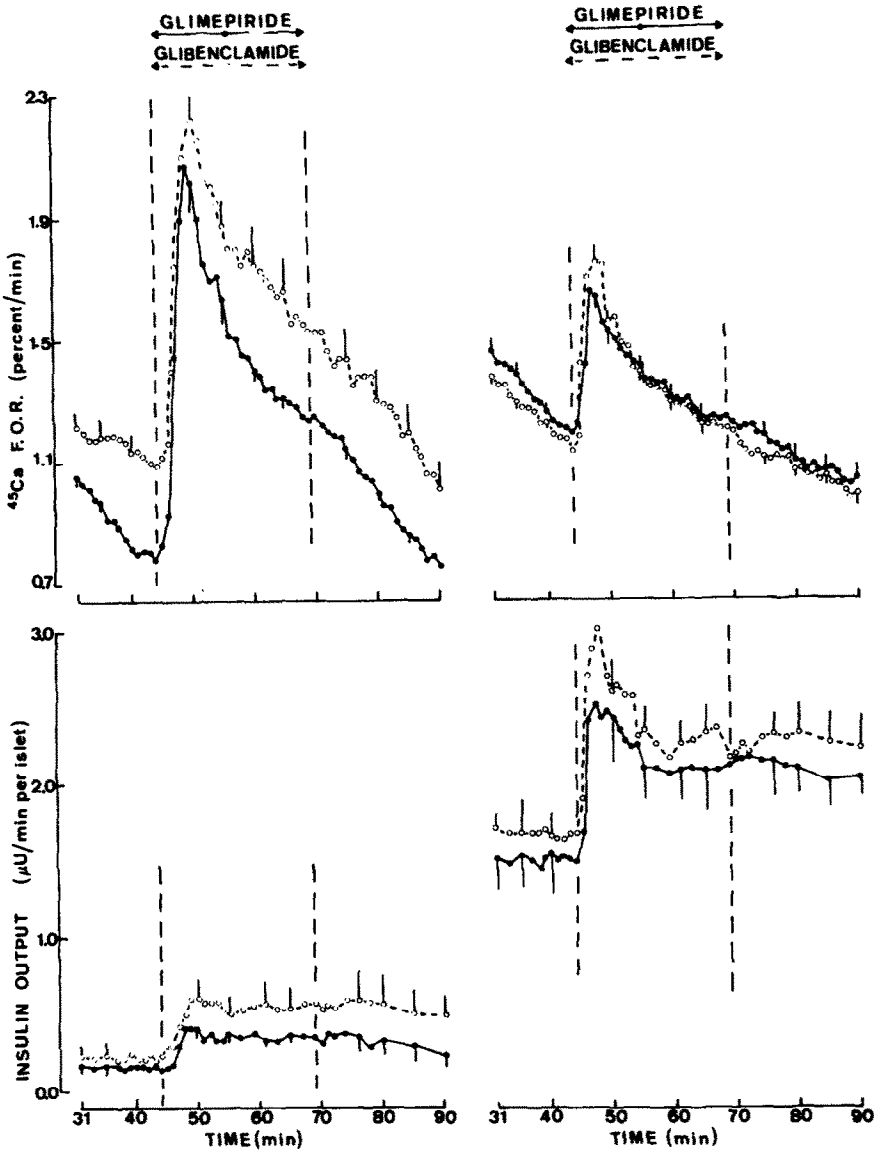

Fig. 1. Effect of either glibenclamide or glimepiride (1.0 μM each) upon ^{45}Ca fractional outflow rate (upper panels) and insulin release (lower panels) from islets perifused for 90 min in the presence of either 5.6 mM D-glucose (left panels) or 16.7 mM D-glucose (right panels). The sulfonylureas were administered from the 45th to 69th min, as indicated by the vertical dotted lines. Mean values (±SEM) refer to four to five individual experiments in each case.

Table 2. Effect of glibenclamide (1.0 μM) and glimepiride (1.0 μM) upon the metabolism of D-glucose (6.0 mM) in pancreatic islets

Tracer	Metabolite	Control	Glibenclamide	Glimepiride
D-[5-^{3}H]Glucose	^{3}HOH	46.9 ± 3.3 (39)*	47.3 ± 3.5 (40)	47.7 ± 2.9 (41)
D-[3,4-^{14}C]Glucose	$^{14}CO_2$	31.6 ± 2.6 (12)	32.9 ± 3.4 (14)	30.8 ± 2.2 (13)
D-[2-^{14}C]Glucose	$^{14}CO_2$	26.2 ± 2.6 (13)	26.4 ± 2.4 (12)	23.7 ± 3.8 (14)
D-[6-^{14}C]Glucose	$^{14}CO_2$	10.1 ± 1.1 (14)	9.1 ± 1.6 (14)	11.5 ± 1.4 (14)
D-[3,4-^{14}C]Glucose	[^{14}C]Amino acids	2.9 ± 0.6 (13)	2.9 ± 0.7 (14)	3.9 ± 0.8 (14)
D-[2-^{14}C]Glucose	[^{14}C]Amino acids	9.0 ± 1.1 (13)	10.8 ± 1.9 (12)	8.2 ± 1.0 (13)
D-[6-^{14}C]Glucose	[^{14}C]Amino acids	15.3 ± 1.5 (14)	14.4 ± 0.9 (14)	13.7 ± 0.9 (14)
D-[3,4-^{14}C]Glucose	Acidic [^{14}C]metabolites	9.9 ± 1.5 (7)	9.0 ± 1.1 (7)	9.5 ± 1.6 (7)
D-[2-^{14}C]Glucose	Acidic [^{14}C]metabolites	12.9 ± 3.2 (7)	13.2 ± 3.6 (7)	12.7 ± 2.2 (7)
D-[6-^{14}C]Glucose	Acidic [^{14}C]metabolites	20.2 ± 1.9 (7)	17.6 ± 2.2 (7)	17.8 ± 1.6 (7)

* All metabolic flows are expressed as pmol of D-glucose equivalent/islet per 120 min.

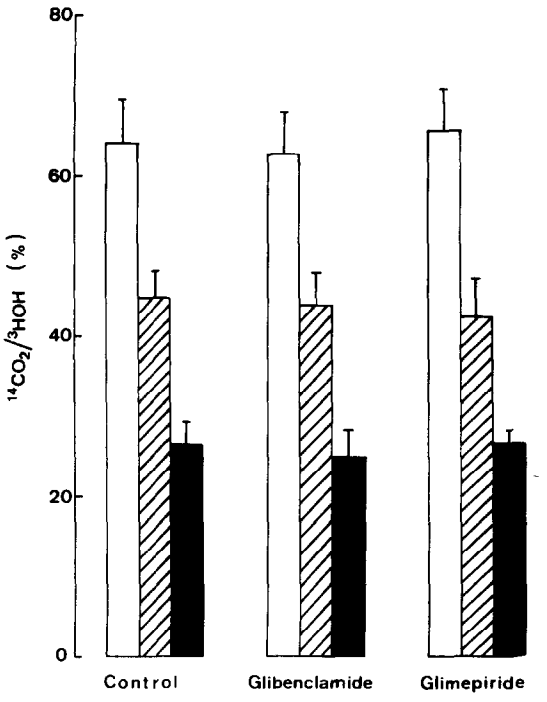

Fig. 2. Ratio between the oxidation of D-[3,4-^{14}C]glucose (open columns), D-[2-^{14}C]glucose (hatched columns) or D-[6-^{14}C]glucose (black columns) and the paired utilization of D-[5-^{3}H]glucose in islets incubated in the absence (control) or presence of glibenclamide (1.0 μM) and glimepiride (1.0 μM). Mean values (±SEM) refer to 12–14 individual observations.

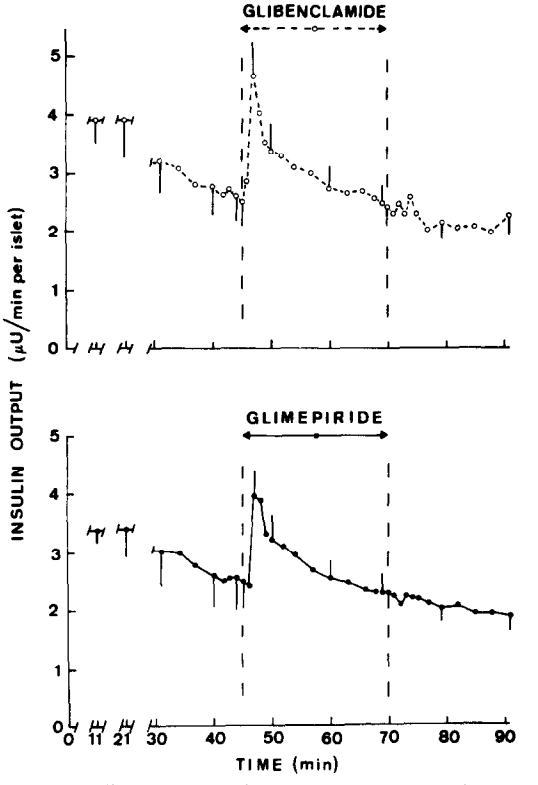

Fig. 3. Insulin output by islets prepared from glucose-infused rats and perifused for 91 min in the presence of 16.7 mM D-glucose. Either glibenclamide or glimepiride (1.0 μM each) was administered from the 46th to 70th min. Mean values (±SEM) refer to six individual experiments in each case.

in ^{45}Ca fractional outflow rate at increasing concentrations of D-glucose. It should be emphasized, however, that the relative magnitude of the drug-induced increase in $^{40}Ca^{2+}$ inflow into the B-cell cannot be judged from data obtained at variable D-glucose concentrations, since the hexose may also modulate, in a concentration-related manner, the extent of $^{45}Ca^{2+}$ outflow provoked in the prelabeled islets by a given increase in $^{40}Ca^{2+}$ inflow [14].

At the highest concentration of D-glucose tested in the present experiments, the rate of insulin release was initially almost two times higher in islets isolated from rats infused for 2 days with a hypertonic solution of D-glucose than in islets prepared from normoglycemic animals. Such a difference is even more remarkable, if consideration is given to the low insulin content (0.59 ± 0.07 mU/islet; N = 9) of the islets prepared from the glucose-infused rats [15]. In the presence of 16.7 mM D-glucose, the sulfonylurea-induced increment in insulin output was as marked in the islets from glucose-infused rats as in control animals. Our findings thus indicate that glibenclamide and glimepiride are able to stimulate insulin release from islets exposed for several days, first *in vivo* and then *in vitro*, to very high concentrations of D-glucose. Even under these conditions, no obvious reversibility of the insulinotropic response to the sulfonylureas was observed within 20 min after their removal from the perifusate.

In islets from euglycemic rats, the tight glucose-dependency of the cationic and secretory responses to glibenclamide or glimepiride could not be accounted for by any obvious effect of these sulfonylureas upon the metabolism of D-glucose in the islets. None of the metabolic parameters examined in the present study in islets exposed to 6.0 mM D-glucose were significantly affected by either glibenclamide or glimepiride. They included the utilization of D-glucose as judged from the

Table 3. Modulation of the secretory response to glibenclamide and glimepiride by D-glucose and SAD

D-Glucose (mM)	SAD (mM)	Control	Glibenclamide (1.0 μM)	Glimepiride (1.0 μM)
—	—	11.3 ± 2.0 (14)*	17.4 ± 1.3 (14)	19.7 ± 1.1 (14)
6.0	—	27.9 ± 1.9 (28)	106.1 ± 6.1 (28)	99.2 ± 5.3 (27)
—	10.0	23.3 ± 2.9 (40)	138.4 ± 3.9 (39)	129.7 ± 4.9 (42)

* All secretory rates are expressed as μU/islet per 120 min.

production of ^{3}HOH from D-[5-^{3}H]glucose, the rate of aerobic glycolysis as estimated from the fate of D-[3,4-^{14}C]glucose, the generation of acetyl residues in the reaction catalysed by pyruvate dehydrogenase as judged from the generation of $^{14}CO_2$ from D-[3,4-^{14}C]glucose, and the oxidation of these residues in the Krebs cycle as measured through the production of $^{14}CO_2$ from both D-[2-^{14}C]glucose and D-[6-^{14}C]-glucose [16]. These findings reinforce the view that the insulinotropic action of hypoglycemic sulfonylureas is not primarily attributable to a facilitation of D-glucose metabolism in the islet cells [17].

The present work also reveals that SAD can be substituted to D-glucose in order to support the insulinotropic action of hypoglycemic sulfonylureas. In fact, the secretory response to glibenclamide or glimepiride was more marked in the presence of SAD than D-glucose, although the succinic acid ester was used at a concentration of slightly lower insulinotropic efficiency than that of the hexose [6]. Since SAD was recently documented to be a potent insulin secretagogue not only *in vitro* but also *in vivo**, it is tempting to propose that, in non-insulin-dependent diabetes, the combined administration of SAD and a hypoglycemic sulfonylurea could induce a much greater secretory response than that evoked by the sole administration of the sulfonylurea. This view is reinforced by the knowledge that, in non-insulin-dependent diabetes, the functional response of the B-cell to D-glucose, including the modulatory role of the hexose upon sulfonylurea-induced insulin release, is preferentially impaired, whilst the insulinotropic action of other nutrient secretagogues, possibly including SAD, appears less severely or little affected [18].

Acknowledgements—This study was supported by grants from the Belgian Foundation for Scientific Medical Research and a grant-in-aid from Hoechst AG (Frankfurt, F.R.G.). The authors are grateful to M. Mahy, J. Marchand, J. Schoonheydt and M. Urbain for technical assistance and C. Demesmaeker for secretarial help.

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