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ENHANCEMENT BY SUCCINIC ACID DIMETHYL ESTER OF INSULIN RELEASE EVOKED BY D-GLUCOSE AND GLIMEPIRIDE IN THE PERFUSED PANCREAS OF NORMOGLYCEMIC AND HYPERGLYCEMIC RATS

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Abstract—The present study deals with the insulinotropic action of the dimethyl ester of succinic acid (SAD), considered as a potential tool for the treatment of non-insulin-dependent diabetes mellitus. In the perfused pancreas prepared from either euglycemic rats or animals first infused for 48 hours with a solution of D-glucose, SAD (10 mM) markedly enhanced insulin output evoked by a high concentration of D-glucose (16.7 mM), whether in the absence or presence of glimepiride (0.5 μM). The succinate ester failed, however, to affect glucagon secretion. Thus, SAD indeed displays favourable attributes for stimulation of insulin release in type 2 diabetes, with emphasis on its insulinotropic efficiency at high concentrations of D-glucose in an animal model of B-cell glucotoxicity.

Key words: perfused pancreas; insulin secretion; succinic acid dimethyl ester

The methyl esters of succinic acid were recently proposed to represent new tools for stimulation of insulin release in non-insulin-dependent diabetes mellitus [1]. These esters display several favourable attributes in such a perspective. First, as their insulinotropic action is attributable to a concerted increase in the supply of succinic acid and acetyl CoA to the Krebs cycle [2], they may bypass site-specific defects of D-glucose transport and metabolism in the B-cell of type 2 diabetics [3]. Second, they stimulate both proinsulin biosynthesis and insulin release, the latter effect being operative both *in vitro* and *in vivo* [4, 5]. Third, they can substitute for D-glucose to enhance the insulinotropic action of hypoglycemic sulfonylureas [6, 7]. Fourth, they are devoid of glucagonotropic action, at least *in vivo* [7]. Last, they protect the B-cell against selected cytotoxic aggressions [1, 8].

The present study concerns three further interrelated aspects of the B-cell secretory response to SAD†, considered as a potential insulinotropic tool in type 2 diabetes. First, it was investigated whether SAD enhances insulin release at a high concentration of D-glucose such as that encountered in diabetic patients. Second, the secretory response to the combination of SAD and glimepiride, a novel hypoglycemic sulfonylurea [9, 10], was explored at the high concentration of D-glucose. Last, these experiments were conducted in pancreases prepared from both control rats and animals which were first infused for 48 hr with a hypertonic solution of D-glucose, the latter procedure being currently used as model of B-cell glucotoxicity [11, 12].

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† Abbreviation: SAD, succinic acid dimethyl ester.

MATERIALS AND METHODS

Animals. Fourteen female Wistar rats (Proefdierencentrum, Heverlee, Belgium) were used in the present study (Table 1). At all times the animals had free access to water and food (AO4, Usine d'alimentation rationnelle, Villemoisson-sur-Orge, France). Seven rats were used as normal controls and seven were rendered hyperglycemic by a 48-hr infusion of D-glucose. The technique for the infusion of D-glucose in unrestrained rats was adapted from Laury *et al.* [13] and described previously [12]. A catheter was inserted under anesthesia in an external jugular vein. After 2 days of recovery, the animals were infused for 48 hr from 8 hr onwards (day 1) with a hypertonic solution of D-glucose (1.67 M, Baxter, Lessines, Belgium) at a rate close to 4.6 mmol D-glucose per hour. In the majority of these animals, the glycemia was measured at approximately 8, 12 and 16 hr of the experimental days by teststrips (Medi-Test, Macherey-Nagel, Düren, Germany) in blood samples obtained from the tail. On the day of the perfusion (day 3), the rats were disconnected from the infusion pump at 9 hr and a 1 mL blood sample was collected from the tail prior to anesthesia for the measurement of the plasma glucose and insulin concentrations. An additional 0.3 mL blood sample was obtained for the estimation of the plasma glucose concentration at the end of the perfusion surgical procedure. In the control rats, the plasma glucose and insulin concentrations were measured on the day of the perfusion at comparable times prior to anesthesia and at the end of the surgical procedure.

Perfusion procedure. The rats were anesthetized with sodium barbital (42 mg/kg, i.p.) and the pancreas was perfused free from all adjacent organs through both the coeliac and superior mesenteric

Table 1. Metabolic and hormonal status of control and 48-hour glucose-infused rats

	Control (N = 7)	Hyperglycemic (N = 7)	P-value
Rat weight (g)	279 ± 15	294 ± 15	NS
Plasma glucose (mM)			
prior to anesthesia	7.1 ± 0.3	16.9 ± 3.1	<0.01
at end of surgery	14.3 ± 1.2	13.6 ± 3.4	NS
Plasma insulin (μU/mL)*	82 ± 12	1047 ± 135	<0.001
Pancreas wet weight (mg)	652 ± 26	555 ± 37	<0.05
Pancreas insulin content (μg)	104.9 ± 6.4	30.2 ± 4.2	<0.001
(μg/g)	163.6 ± 14.6	54.3 ± 5.9	<0.001
Pancreas glucagon content (μg)	5.7 ± 0.7	7.5 ± 0.9	NS
(μg/g)	8.5 ± 0.7	13.5 ± 1.3	<0.01
Insulin/glucagon ratio (M)	11.9 ± 1.9	2.5 ± 0.4	<0.001
Flow rate (mL/min)	1.48 ± 0.02	1.41 ± 0.06	NS
Perfusion pressure (mmHg) 20 min	27.4 ± 1.8	25.9 ± 3.1	NS
85 min	26.3 ± 1.4	24.7 ± 3.3	NS

* Measurement made in the sample taken prior to anesthesia.
NS, not significant

arteries as previously described [14, 15]. The basal perfusion medium contained the following salts (in mM): NaCl, 118.5; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4$, 0.6; $CaCl_2$, 1.0; $NaHCO_3$, 25. It was supplemented with dextran (40 g/L clinical grade, Sigma, St Louis, MO, U.S.A.), bovine serum albumin (5 g/L, fraction V, RIA grade, Sigma) and D-glucose (16.7 mM), and continuously gassed with a mixture of O_2 and CO_2 (95:5) which resulted in a pH of approximately 7.4. The perfusion medium was infused into the aorta using a peristaltic pump (Minipuls 2, Gilson, Villiers-le-Bel, France). The insulinotropic agents SAD (final concentration 10 mM, succinic acid dimethyl ester, Sigma, St Louis, MO, U.S.A.) and glimepiride (final concentration 0.5 μM) were administered, either alone or in combination, through side-arm syringes containing saline and working at a flow rate of 0.075 mL/min (Unita I infusion pump, Braun, Melsungen, Germany). Glimepiride was first dissolved in DMSO (Me_2SO_4, final concentration in the perfusion medium 0.01%, v/v). The pressure was recorded throughout the perfusion with a blood pressure monitor (Palmer, London, U.K.). After a 20 min equilibration period, samples of the pancreatic effluent were collected from the portal vein at 1 min intervals in chilled tubes containing 0.15 mL of aprotinin (10,000 kallikrein inhibitor units/mL, Trasylol, Bayer, Brussels, Belgium) and EDTA (32 mM). At the end of the perfusion, the pancreas was dissected free from fat and lymph nodes, weighed, and extracted using acidified ethanol [16] and a mechanical homogenizer (Talboys Engineering Corporation, Montrose, PA, U.S.A.). The effluent samples and the pancreas extracts were all stored at −25° until time of assay.

Analytical procedures. The plasma glucose concentration was estimated by a hexokinase method (Sigma Diagnostics). The measurement of glucagon and insulin in the effluent samples and pancreatic extracts was performed as previously described, using our own antisera [17, 18].

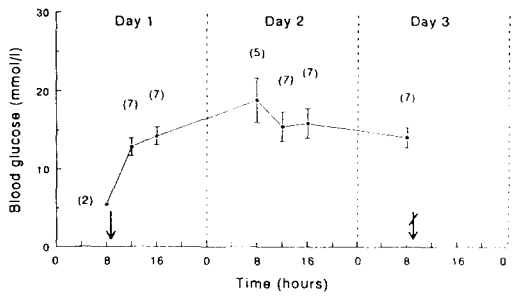

Fig. 1. Glycemic pattern in rats infused for 48 hr with glucose. Values (±SEM) refer to the number of determinations indicated in parentheses. The arrows at the bottom indicate the onset and end of the glucose infusion.

Presentation of results. All results are expressed as means ± SEM. Integrated insulin and glucagon responses were computed from the areas under the curves. Statistical analyses were conducted using Student's two-tailed *t*-test for unpaired data.

RESULTS

In vivo *parameters*

In the glucose-infused rats (Fig. 1), the glycemia increased from a basal value of 5.4 ± 0.1 mM (at 8 hr on day 1, N = 5) to a plateau value in the range between 12.8 ± 1.1 mM (at 12 hr on day 1, N = 7) and 18.8 ± 2.8 mM (at 8 hr on day 2, N = 5). In the morning of the last day, the glycemia averaged 14.0 ± 1.3 mM (at 8 hr on day 3, N = 7). It still amounted to 18.1 ± 2.6 mM around 9 hr, immediately after the rats were disconnected from the infusion pump and prior to anesthesia (N = 7). At a comparable time, i.e. at about 9 hr and prior to anesthesia, the plasma glucose concentration was significantly higher in the glucose-infused than in the

Table 2. Effects of glimepiride (0.5 μM), SAD (10 mM), or the combination of these two stimuli on the secretion of insulin and glucagon from the pancreas of control and hyperglycemic rats perfused in the presence of 16.7 mM glucose

		Control	Hyperglycemic	P-value
Insulin output (ng/min)				
Basal output	(Figs 2, 3, 20–25 min)	63 ± 9 (7)	96 ± 19 (7)	NS
Glimepiride	(Fig. 2, 26–50 min)	116 ± 26 (4)	160 ± 28 (4)	<0.05
Glimepiride + SAD	(Fig. 2, 51–75 min)	317 ± 59 (4)	251 ± 24 (4)	NS
SAD	(Fig. 3, 26–50 min)	235 ± 58 (3)	156 ± 63 (3)	NS
SAD + glimepiride	(Fig. 3, 51–75 min)	420 ± 88 (3)	167 ± 55 (3)	NS
Glucagon output (pg/min)				
Basal output	(Figs 4, 5, 20–25 min)	97 ± 21 (7)	100 ± 12 (7)	NS
Glimepiride	(Fig. 4, 26–50 min)	72 ± 7 (4)	107 ± 19 (4)	NS
Glimepiride + SAD	(Fig. 4, 51–75 min)	67 ± 5 (4)	105 ± 16 (4)	NS
SAD	(Fig. 5, 26–50 min)	98 ± 23 (3)	81 ± 17 (3)	NS
SAD + glimepiride	(Fig. 5, 51–75 min)	80 ± 14 (3)	91 ± 23 (3)	NS

NS, not significant.

control rats (Table 1). In addition to the marked hyperglycemia, the insulin concentration in the plasma was considerably higher in the glucose-infused than control animals (Table 1). The plasma glucose concentration decreased slightly upon anesthesia and surgery in the hyperglycemic rats to a mean value of 13.6 ± 3.4 mM (Table 1). The latter value was comparable to that seen at the same time in the normal animals in which, as usual, the plasma glucose concentrations increased upon anesthesia and surgery (Table 1). Both groups of rats displayed a comparable body weight on the day of perfusion (Table 1).

Perfusion parameters

There was no difference between the mean flow rates measured during perfusion of the pancreas from either hyperglycemic or control rats (Table 1). The perfusion pressure values were comparably stable throughout the experiments (20–85 min), and in the same range for both groups of animals (Table 1).

Weight, insulin and glucagon content of the pancreas

The wet weight of the pancreas was significantly decreased in the hyperglycemic rats (Table 1). The insulin content of the pancreas was also severely diminished in the hyperglycemic animals, whether expressed in terms of total content (μg) or relative to the weight of the pancreas (μg/g). By contrast, the glucagon content of the pancreas was increased in the hyperglycemic rats, such an increase being highly significant when expressed relative to the weight of the pancreas (Table 1). The molar ratio of insulin over glucagon was thus markedly decreased in the hyperglycemic rats.

Insulin output

After 20 to 25 min exposure to D-glucose (16.7 mM), the output of insulin was not significantly different in control and hyperglycemic rats (Table 2), averaging respectively 62.8 ± 9.4 and 96.3 ± 19.5 ng/min (N = 7 in both cases). Relative to the insulin content of the pancreas, however, the output of insulin was about 6 times higher in hyperglycemic rats (3.8 ± 1.1 10^{-3} min^{-1}) than control animals (0.6 ± 0.1 10^{-3} min^{-1}).

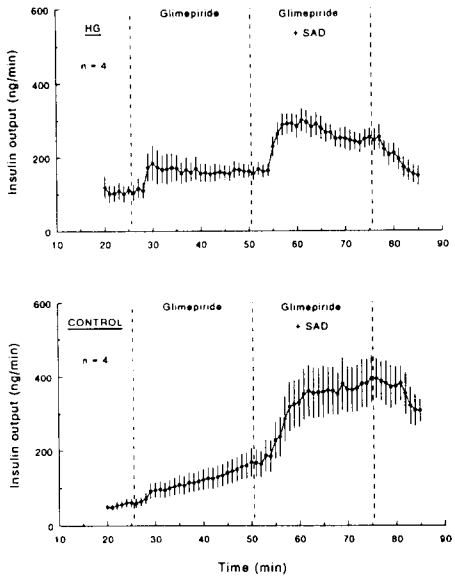

Fig. 2. Effects of glimepiride (0.5 μM, 25–75 min), and SAD (10 mM) superimposed on glimepiride (50–75 min), upon the secretion of insulin from the perfused pancreas of control (lower panel) and 48-hr glucose-infused rats (HG, upper panel). Glucose (16.7 mM) was present throughout the perfusions. Mean values (±SEM) are shown together with the number of experiments performed in each group of animals.

The administration of glimepiride from 26 to 51 min caused a sizeable increase in insulin output in both control and hyperglycemic rats (Fig. 2). Relative to the paired reference output (20–25 min), the glimepiride-induced increment in output appeared somewhat greater in control rats (+105.9 ± 16.2%; N = 4) than in hyperglycemic

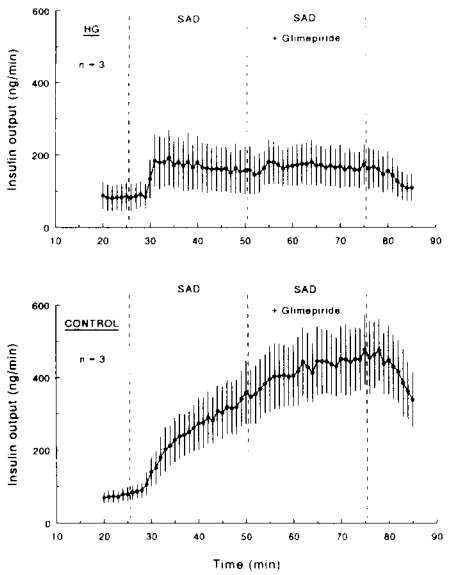

Fig. 3. Effects of SAD (10 mM, 25–75 min), and glimepiride (0.5 μM) superimposed on SAD (50–75 min), upon the secretion of insulin from the perfused pancreas of control (lower panel) and 48-hr glucose-infused rats (HG, upper panel). Concentration of glucose in the perfusate and presentation as in Fig. 2.

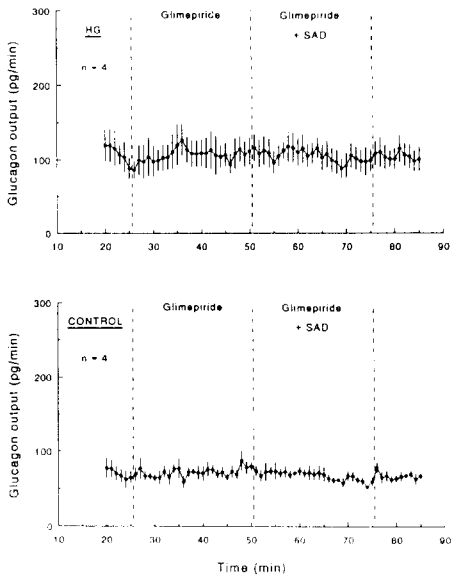

Fig. 4. Effects of glimepiride (0.5 μM, 25–75 min), and SAD (10 mM) superimposed on glimepiride (50–75 min), upon the secretion of glucagon from the perfused pancreas of control (lower panel) and 48-hr glucose-infused rats (HG, upper panel). The experiments correspond to those illustrated for insulin in Fig. 2.

animals (+59.4 ± 13.8%; N = 4). Such a difference was also observed when glimepiride was administered from 51–76 min in the concomitant presence of D-glucose and SAD (Fig. 3). In such a case, the sulfonylurea-induced increment in insulin output above the paired mean reading recorded between 26 and 51 min and expressed relative to the reference initial value (20–25 min) averaged 267.9 ± 62.5% in control rats but failed to achieve statistical significance (+30.2 ± 26.7%; N = 3) in hyperglycemic animals. The data recorded in the control rats indicate that the presence of SAD potentiated the insulinotropic action of glimepiride whether expressed in absolute values (185 ± 47 ng/min in the presence of SAD as compared with 62 ± 16 ng/min in the absence of SAD) or relative to the initial reference value (268 ± 62% in the presence of SAD as compared with 106 ± 16% in the absence of SAD). It should be stressed, however, that the two series of values were computed over distinct periods during perfusion.

The administration of SAD always caused a dramatic increase in insulin output, whether in control or hyperglycemic rats and whether before or after introduction of glimepiride. The absolute value for the SAD-induced increment in insulin output was of comparable magnitude when measured prior or after glimepiride administration. Pooling all available data, it averaged 183.9 ± 25.1 and 83.5 ± 17.8 ng/min (N = 7 in both cases) in control and hyperglycemic rats respectively. Expressed relative to the paired initial reference output (20–25 min), the latter increments yielded mean values of 309.1 ± 37.2% in control rats as distinct ($P < 0.005$) from only 109.9 ± 30.1% in hyperglycemic rats.

Glucagon output

Between 20 and 25 min, the output of glucagon averaged 97.2 ± 21.3 and 100.2 ± 11.9 pg/min (N = 7 in both cases) in control and hyperglycemic rats, respectively (Table 2). Neither SAD nor glimepiride exerted any obvious effect upon glucagon release (Figs 4 and 5).

DISCUSSION

The present experiments afford in essence five pieces of information.

First, they indicate that SAD and/or glimepiride exert no glucagonotropic action, at least at a high concentration of D-glucose. The mono- and dimethyl ester of succinic acid also fail to affect the plasma concentration of glucagon when tested *in vivo* in normoglycemic rats [7].

Second, SAD caused a marked enhancement of insulin release from pancreases already exposed for 25 min or more to a high concentration of D-glucose close to that required to evoke a maximal secretory response to the hexose. A comparable situation had already been documented in isolated islets over 90 min incubation [4].

Third, the efficiency of SAD as an insulinotropic agent was equally marked in the sole presence of D-glucose and in the concomitant presence of the hexose and glimepiride.

Fourth, in the pancreas from normoglycemic rats, SAD potentiated the B-cell secretory response to

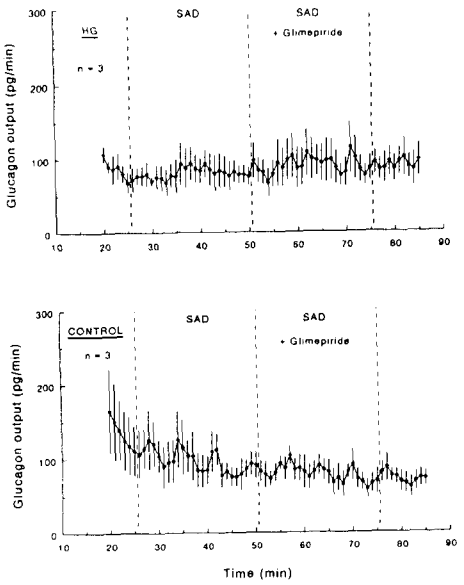

Fig. 5. Effects of SAD (10 mM, 25–75 min), and glimepiride (0.5 μM) superimposed on SAD (50–75 min), upon the secretion of glucagon from the perfused pancreas of control (lower panel) and 48-hr glucose-infused rats (HG, upper panel). The experiments correspond to those illustrated for insulin in Fig. 3.

glimepiride. A comparable behaviour was previously documented in islets incubated in the absence of D-glucose [6] and in experiments conducted *in vivo* in normoglycemic rats [7]. No potentiation of sulfonylurea-induced insulin release was here observed in the pancreas of hyperglycemic rats. It should be acknowledged, however, that, relative to the insulin content of the pancreas, the secretory rate had already reached extremely high values in the pancreas of hyperglycemic rats exposed to both D-glucose and SAD prior to the administration of glimepiride. In the sole presence of D-glucose, the ratio between insulin output and content was indeed about 6 times higher in hyperglycemic than control rats; in the former animals, such a ratio was almost doubled (189 ± 36%; N = 3) by the further administration of SAD.

Fifth, and most importantly, SAD proved quite efficient in stimulating insulin release from the pancreas of rats first infused for 48 hr with a hypertonic solution of D-glucose. In this experimental model of B-cell glucotoxicity, the B-cell displays a paradoxical secretory behaviour in response to rapid changes in D-glucose concentration [11, 12]. Yet SAD was still able to provoke a rapid and sustained stimulation of insulin release in the pancreas of hyperglycemic rats. This situation is reminiscent of that recently documented in response to another non-glucidic nutrient secretagogue, namely 2-keto-isocaproic acid [19]. Taken as a whole, these findings support the view that the paradoxical immediate secretory response to D-glucose seen both in animal models of type 2 diabetes and patients affected by this disease [20, 21] is attributable to a paradoxical change in glycolytic flux [12] rather than an altered coupling between ATP availability and more distal events in the secretory sequence.

In conclusion, the present study documents that SAD displays suitable attributes for stimulation of insulin release in type 2 diabetes, with emphasis on its insulinotropic action at high concentrations of D-glucose whether in the presence or absence of a hypoglycemic sulfonylurea and whether in the pancreas from euglycemic or hyperglycemic animals.

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