ELSEVIER

European Journal of Pharmacology 344 (1998) 87–93

ejp

Comparison between the insulinotropic potential of ten new esters of succinic acid

Laurence Ladrière [a], Aouatif Laghmich [a], Francine Malaisse-Lagae [a], Heinz Dannacher [b], Fredrik Björkling [b], Willy J. Malaisse [a,*]

[a] *Laboratory of Experimental Medicine, Brussels Free University, 808 Route de Lennik, 1070 Brussels, Belgium*
[b] *Leo Pharmaceutical Products, 2750 Ballerup, Denmark*

Received 1 May 1997; revised 28 November 1997; accepted 2 December 1997

Abstract

Selected esters of succinic acid are currently under investigation as insulinotropic tools for the treatment of non-insulin-dependent diabetes mellitus. The aim of the present study was to investigate, in isolated rat pancreatic islets, the insulin secretory response to ten novel esters of succinic acid. According to six different methods of comparison, the following hierarchy in insulinotropic potential was established: 4-*tert*-butyl-succinate ≤ glycerol-1,2-dimethylsuccinate-3-hydrogenosuccinate ≤ threitol-3-succinoyl-1,2,4-trimethylsuccinate ≤ ethanediol-1,2-diethylsuccinate ≤ glycerol-1,2-dimethylsuccinate ≤ glycerol-3-hydroxy-1,2-dimethylsuccinate ≤ arabitol-5-hydroxy-1,2,3,4-tetramethylsuccinate ≤ threitol-1,2,4-trimethylsuccinate ≤ ethanediol-1,2-dimethylsuccinate < propanediol-1,2-dimethylsuccinate. There was a close correlation ($r = 0.823$) between the insulinotropic potential and the minimal effective concentration, which ranged between the extreme values of 10 μM and 2.5 mM. In the presence of the esters, the concentration–response relationship for glucose-stimulated insulin release was changed from its typically sigmoidal shape to a hyperbolic pattern, with most agents enhancing insulin output at a low hexose concentration (2.8 mM) but failing to do so at a high glucose level (16.7 mM). Highly potent insulinotropic esters have several advantages over other antidiabetic agents in clinical use. © 1998 Elsevier Science B.V.

Keywords: Succinic acid ester; Pancreatic islet; Insulin secretion

1. Introduction

A novel approach to the treatment of non-insulin-dependent diabetes mellitus is to use non-glucidic nutrients (Malaisse, 1994). For instance, selected esters of succinic acid are presently under investigation as possible tools for stimulation of insulin release in type 2 diabetes (Malaisse, 1995). As already underlined in prior reviews (Malaisse, 1994, 1995), this approach offers several potential advantages. First, non-glucidic nutrients may be able to bypass those site-specific defects of glucose transport and metabolism currently held responsible for a preferential impairment of the secretory response to D-glucose in diseased pancreatic islet B-cells. Second, at variance with all other insulinotropic agents currently used or proposed as antidiabetic tools, e.g. hypoglycemic sulfonylureas and meglitinide analogs, non-glucidic nutrients stimulate both proinsulin biosynthesis and insulin release. Third, they have been shown to protect pancreatic islet B-cells against cytotoxic events that might participate in the pathogenesis of the diabetic state. Last, they inhibit glucagon secretion (Leclercq-Meyer and Malaisse, 1996). The choice of succinic esters as non-glucidic nutrients is motivated mainly by the two following considerations. First, at variance with unesterified succinic acid, these esters penetrate efficiently into the islet cells and exert an obvious insulinotropic action (Malaisse et al., 1993). Second, of several esters of carboxylic nutrients that are either intermediates of the Krebs cycle or their precursors (Sener et al., 1994; Malaisse et al., 1996), the esters of succinic acid appear to be the most potent insulinotropic tools.

For most esters investigated so far, one potential limitation of this therapeutic strategy is the high doses required to stimulate insulin release, with resulting enhancement of hepatic gluconeogenesis (Zhang et al., 1994).

The aim of the present study is to investigate the insulinotropic action of ten new esters of succinic acid, in

* Corresponding author. Tel.: +32-2-5556238; fax: +32-2-5556239; e-mail: malaisse@med.ulb.ac.be

the perspective of designing molecules of high secretory potency.

2. Materials and methods

The ten esters examined in the study (Fig. 1) were 4-*tert*-butyl-succinate (TB-1S), ethanediol-1,2-dimethylsuccinate (G-2MS), ethanediol-1,2-diethylsuccinate (G-2ES), propanediol-1,2-dimethylsuccinate (P_2-2MS), glycerol-1,2-dimethylsuccinate (Gl-2MS), glycerol-3-hydroxy-1,2-dimethylsuccinate (P_4-2MS), glycerol-1,2-dimethylsuccinate-3-hydrogenosuccinate (Gl-2MS,S), L-threitol-1,2,4-trimethylsuccinate (Th-3MS), D-arabitol-5-hydroxy-1,2,3,4-tetramethylsuccinate (Pe-4MS) and L-threitol-3-succinoyl-1,2,4-trimethylsuccinate (Th-3MS,S). They are ranked in this enumeration according to the number of succinyl residues (S) in each molecule. They were prepared by direct esterification of the corresponding polyol by the desired succinic acid derivative using previously reported procedures (Patent application, UK, 9612331.0, 1996).

All experiments were conducted with pancreatic islets isolated by the collagenase technique (Malaisse-Lagae and Malaisse, 1984) from fed female Wistar rats (Proefdierencentrum, Heverlee, Belgium). For the measurement of insulin release, groups of 8 islets each were incubated for 90 min at 37°C in 1.0 ml of a bicarbonate-buffered medium (Malaisse-Lagae and Malaisse, 1984). The esters of succinic acid were incorporated into the incubation medium from stock solutions prepared in dimethyl sulfoxide. The same concentration of the solvent (1%, v/v) was present in control (no ester) and test media. At this concentration, dimethyl sulfoxide fails to affect islet function (Levy et al., 1976).

All results are expressed as mean values (±S.E.M.), together with either the number of individual determinations (n) or degree of freedom. The statistical significance of differences between mean values was assessed using Student's t-test. Except for the data illustrated in Fig. 2,

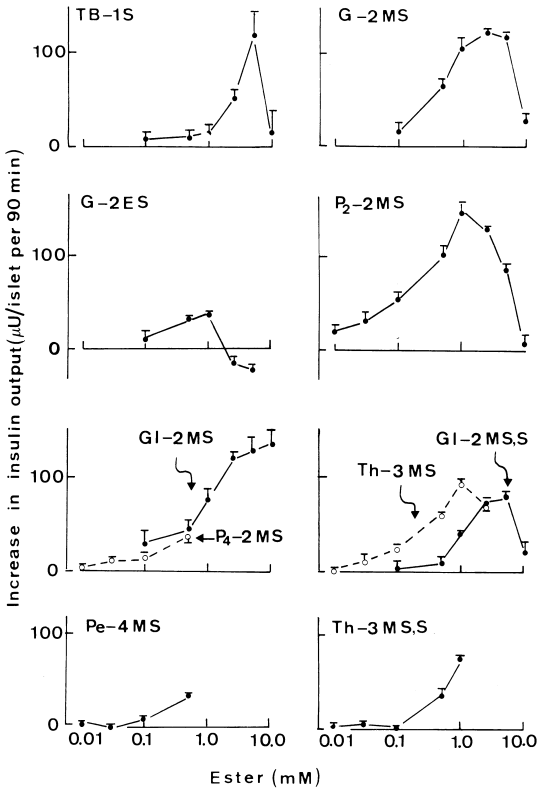

Fig. 2. Concentration–response relationships for the increase in insulin release evoked by the esters of succinic acid in islets incubated in the presence of 7.0 mM D-glucose. The concentrations of the esters are ranged on a logarithmic scale. Mean values (±S.E.M.) refer to 17–91 individual measurements (mean±SD: 31±17 measurements; $n=57$). The increase in insulin output was calculated after subtraction of the mean control value (no ester) found in the same experiment(s). However, the data obtained at different concentrations of the esters were often derived from separate experiments.

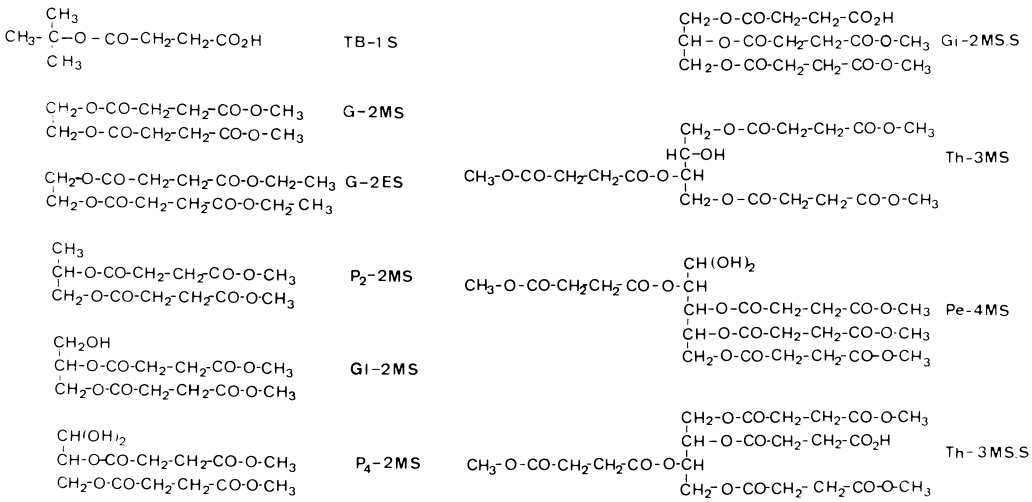

Fig. 1. Chemical structure of the ten esters examined in this report.

the comparison between mean values is restricted to measurements made in the same experiment(s).

3. Results

3.1. Secretory response to the esters at 2.8 mM D-glucose

The output of insulin recorded in the presence of 2.8 mM D-glucose averaged 17.3 ± 1.1 μU/islet per 90 min ($n = 202$). In the experiments conducted at D-glucose concentrations of 2.8, 11.1 and 16.7 mM, the esters were used at concentrations either close to the limit of solubility or yielding a near maximal secretory response in islets exposed to 7.0 mM D-glucose (see below). Only two out of the ten esters examined in the present study failed to significantly affect insulin release at the low glucose level (Table 1). Such was the case with P_4-2MS, which was tested at a concentration of only 0.1 mM and Th-3MS,S, which was used at 2.0 mM. Gl-2MS,S (2.5 mM) only increased insulin secretion by about 50% ($P < 0.005$). Four other esters, namely Pe-4MS (0.5 mM), G-2ES (1.0 mM), Th-3MS (1.0 mM) and TB-1S (2.5 mM), approximately doubled the secretory rate. Gl-2MS was a more effective secretagogue than Gl-2MS,S and TB-1S, all three esters being tested at 2.5 mM. Last, G-2MS and P_2-2MS (also 2.5 mM each) appeared to be the most potent insulinotropic agents, the output of insulin recorded at the low glucose concentration in the presence of these two esters being close to half the secretory response evoked by 16.7 mM D-glucose in the same experiments.

3.2. Secretory response to the esters at 7.0 mM D-glucose

At 7.0 mM D-glucose, the rate of insulin release averaged 60.6 ± 1.9 μU/islet per 90 min ($n = 516$). The concentration–response relationship for the insulinotropic action of the esters was examined at this hexose concentration (Fig. 2).

Table 2
Insulin release (μU/islet per 90 min) from islets incubated with 7.0 mM D-glucose in the absence or presence of 3 selected esters, tested in two separate series of experiments at either 2.5 or 5.0 mM

Ester	2.5 mM	5.0 mM
Nil	39.7 ± 2.9 (29)	30.7 ± 2.9 (31)
G-2MS	169.6 ± 5.4 (29)	169.6 ± 6.5 (28)
G-2ES	60.4 ± 9.1 (8)	27.2 ± 2.4 (30)
P_2-2MS	168.5 ± 8.7 (28)	75.4 ± 5.8 (29)

Six different comparisons were used to establish the insulinotropic potency of the esters, relative to one another. First, the release of insulin measured in islets incubated with 7.0 mM D-glucose in the presence of a given concentration of each ester was used to rank the different molecules. Such a ranking was made for three concentrations of the ester, namely 0.5 mM (10 esters: ranking from 0.5 to 5.0), 1.0 mM (8 esters: ranking from 0.8 to 6.2) and 2.0–2.5 mM (6 esters: ranking from 1.0 to 6.0). To this end, all available individual measurements, usually obtained in separate experiments, were pooled for each concentration of each drug. At the highest concentration of the ester (2.5 mM), two esters were excluded from the comparison because the mean output of insulin was significantly lower than that recorded at 1.0 mM. An unexpected decrease in insulin release was indeed often observed when the concentration of the ester was raised above the value yielding the highest secretory rate. This is illustrated in Table 2, which compares 3 selected esters tested in two separate series of experiments at either 2.5 or 5.0 mM. When the concentration–response relationship for each individual ester was explored within the same experiment, 6 cases were identified in which there was a decrease in secretory rate at high concentrations of the compound (Table 3). Relative to the values listed in Table 3, a further decrease in secretory rate was observed when the concentration of G-2ES was raised from 2.5 to 5.0 mM and that of P_2-2MS from 5.0 to 10.0 mM. For instance, in the latter case, the output of insulin at 10.0 mM averaged 36.8 ±

Table 1
Insulin release (μU/islet per 90 min) from islets incubated at increasing concentrations of D-glucose in the absence or presence ([a]) of succinic acid esters

Ester (mM)	D-glucose (mM)				
	2.8	2.8[a]	7.0[a]	16.7	16.7[a]
TB-1S (2.5)	23.4 ± 2.3 (28)	54.7 ± 7.3 (29)	109.7 ± 6.3 (19)	251.4 ± 11.8 (28)	235.6 ± 12.5 (29)
G-2MS (2.5)	27.5 ± 3.3 (20)	112.7 ± 5.6 (41)	192.0 ± 6.2 (41)	218.1 ± 13.6 (20)	236.6 ± 10.7 (41)
G-2ES (1.0)	14.4 ± 4.9 (20)	28.9 ± 3.2 (20)	153.4 ± 11.6 (20)	322.3 ± 12.7 (13)	218.8 ± 16.0 (18)
P_2-2MS (2.5)	22.5 ± 6.4 (20)	114.3 ± 3.2 (20)	249.5 ± 11.4 (20)	247.3 ± 14.5 (20)	252.9 ± 12.2 (20)
Gl-2MS (2.5)	14.8 ± 3.1 (20)	73.5 ± 4.0 (20)	154.0 ± 11.5 (20)	235.9 ± 15.2 (20)	233.5 ± 15.4 (20)
P_4-2MS (0.1)	22.6 ± 3.0 (15)	22.6 ± 3.0 (16)	96.0 ± 16.2 (20)	219.2 ± 17.7 (20)	216.1 ± 13.8 (18)
Gl-2MS,S (2.5)	12.7 ± 1.4 (20)	18.6 ± 1.3 (20)	112.2 ± 7.7 (20)	202.7 ± 17.1 (17)	181.9 ± 19.2 (19)
Th-3MS (1.0)	9.4 ± 1.3 (20)	18.9 ± 1.4 (20)	134.0 ± 11.7 (20)	239.4 ± 15.9 (19)	191.6 ± 11.9 (20)
Pe-4MS (0.5)	13.3 ± 2.6 (19)	30.6 ± 2.8 (20)	74.2 ± 5.5 (20)	139.9 ± 10.1 (20)	119.5 ± 10.1 (20)
Th-3MS,S (2.0)	13.1 ± 1.8 (20)	14.9 ± 1.7 (20)	97.5 ± 9.4 (19)	290.2 ± 14.8 (15)	232.7 ± 14.7 (16)

Table 3
Insulin release (μU/islet per 90 min) from islets incubated with 7.0 mM D-glucose

Ester	Optimal concentration		Higher concentration		Higher/optimal concentration	
	mM	insulin output	mM	insulin output	secretory ratio[a] (%)	*P*
TB-1S	5.0	168.0 ± 26.5 (23)	10.0	61.6 ± 24.6 (23)	37.1 ± 19.6 (40)	< 0.005
G-2MS	5.0	188.0 ± 18.2 (19)	10.0	131.5 ± 14.1 (19)	69.0 ± 6.0 (34)	< 0.001
G-2ES	1.0	107.4 ± 5.4 (40)	2.5	42.6 ± 4.2 (39)	39.9 ± 6.0 (71)	< 0.001
P_2-2MS	1.0–2.5	213.5 ± 9.2 (40)	5.0	166.0 ± 9.1 (20)	78.3 ± 6.4 (56)	< 0.005
Gl-2MS,S	5.0	177.3 ± 8.2 (19)	10.0	122.2 ± 11.0 (20)	69.6 ± 8.0 (35)	< 0.001
Th-3MS	1.0	146.9 ± 6.5 (23)	2.5	124.8 ± 4.5 (21)	86.3 ± 5.6 (41)	< 0.02

[a]The secretory ratio at higher/optimal concentration of the ester was calculated by comparison of data recorded in the same experiments; the S.E.M. for such a ratio takes into account the spread of individual measurements at each ester concentration. The degrees of freedom for the secretory ratio are in parentheses.

8.4% (d.f. = 35) of that found in the same experiments in islets exposed to 5.0 mM P_2-2MS (Fig. 2).

The above-mentioned data obtained for the three concentrations of each ester were used to establish a mean concentration-related ranking of all molecules. This ranking is illustrated on the abscissa of Fig. 3.

The next comparison was based on the ratio between the output of insulin recorded for 7.0 mM D-glucose in the presence of each ester and the paired value obtained in the same experiments for 16.7 mM D-glucose without the esters (Table 1). The ratios found for the six esters tested at comparable concentrations (2.0–2.5 mM) were ranked from 1.0 to 6.0 in order of increasing relative insulinotropic activity. The ratio found for the four remaining esters, tested at lower concentrations (0.1 to 1.0 mM), happened not to differ significantly from one another, ranging between the extreme values of 40.9 ± 7.4% (d.f. = 36) and 56.1 ± 8.3% (d.f. = 35) in the case of P_4-2MS (0.1 mM) and Th-3MS (1.0 mM), respectively. By comparison with two esters (TB-1S and Gl-2MS,S) tested at a concentration of 2.5 mM and yielding comparable ratios, it was then possible to ascribe an insulinotropic score to the four molecules tested in the 0.1–1.0 mM range according to the concentration used in these experiments.

The next comparison was based on the same approach defined, except that the primary data under consideration

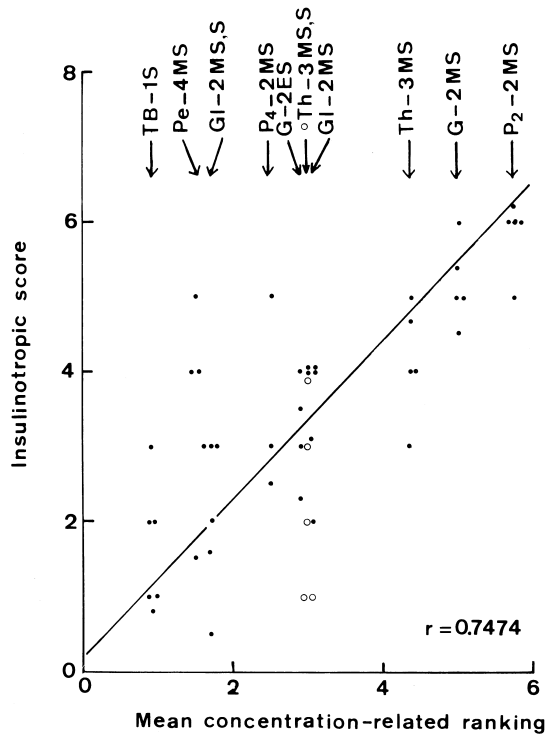

Fig. 3. Correlation (r) between the individual insulinotropic scores obtained for each ester in 6 different comparisons and the corresponding mean ranking derived from measurement of insulin release from islets incubated with 3 concentrations of the drugs (0.5, 1.0 and 2.0–2.5 mM) in the presence of 7.0 mM D-glucose. The oblique line was calculated by regression analysis.

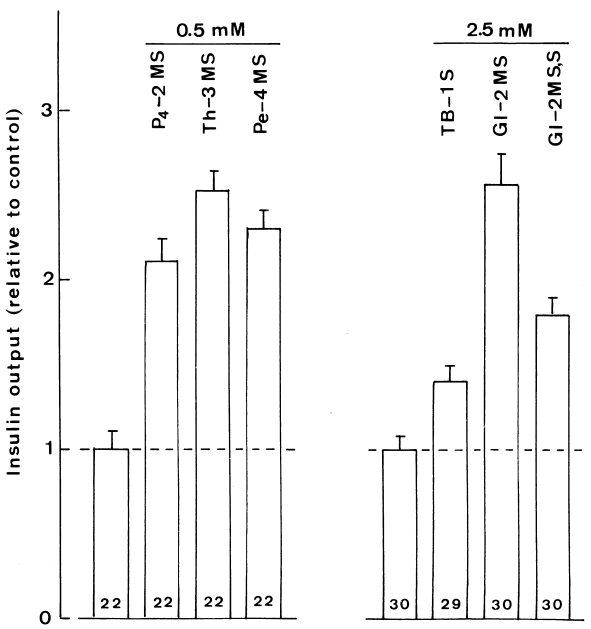

Fig. 4. Mean values (±S.E.M.) for the release of insulin from islets exposed to 7.0 mM D-glucose in the presence or absence of selected esters tested at either 0.5 mM (left panel) or 2.5 mM (right panel). Two separate series of experiments were performed. The horizontal dashed lines refer to the mean control values recorded in the presence of D-glucose alone and taken as reference unit. The number of individual measurements is indicated at the bottom of each column.

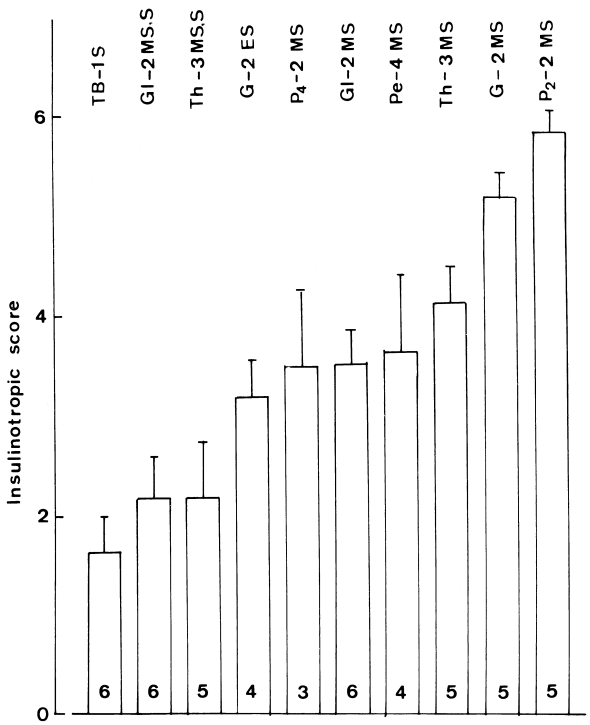

Fig. 5. Mean values (±S.E.M.) for the insulinotropic score of each ester in 6 different comparisons. The drugs are ranged, from left to right, in order of increasing insulinotropic potency. The number of comparisons for each ester is indicated at the bottom of the columns.

now referred to the effect of the esters in islets incubated in the presence of 2.8 mM D-glucose (Table 1). In this case, the three esters tested in the 0.5 to 1.0 mM range (Pe-4MS, G-2ES and Th-3MS) were graded by reference to the ranking of TB-1S (tested at a 2.5 mM concentration), because these four esters virtually all doubled the paired control value for insulin output, as measured in the presence of 2.8 mM D-glucose alone. The ester P_4-2MS was excluded from this comparison because it failed to affect insulin secretion at the low hexose level.

The last comparison was based on two separate series of observations, in each of which the secretory responses to three selected esters, tested at the same concentration in islets exposed to 7.0 mM D-glucose, were compared within the same experiments (Fig. 4). The insulinotropic scores derived from these experiments were adjusted by reference to the scale established in the comparison in which the response to 7.0 mM D-glucose plus ester was compared to that elicited by 16.7 mM glucose alone (see above).

Fig. 3 documents the correlation ($P < 0.001$) found between the insulinotropic scores established in the six comparisons and the mean concentration-related ranking. Fig. 5 illustrates the eventual ordering of the ten esters according to their mean insulinotropic score.

The minimal effective concentrations ranged from 10 μM in the case of P_2-2MS to 2.5 mM in the case of TB-1S, with in-between values of 0.1 mM for P_4-2MS and Th-3MS, 0.5 mM for G-2MS, G-2ES, Gl-2MS, Pe-4MS and Th-3MS,S and 1.0 mM for Gl-2MS,S (Table 4). There was a significant correlation ($r = 0.7526$; $P < 0.02$) between the ranking of the ten esters according to either these values or the mean insulinotropic score (Fig. 5).

Table 4
Minimal effective concentration for the insulinotropic action of succinic acid esters in islets incubated in the presence of 7.0 mM D-glucose

Ester	mM	Increase in insulin output[a]	P
TB-1S	2.5	49.5±9.9 (167)	< 0.001
G-2MS	0.5	65.7±9.5 (32)	< 0.001
G-2ES	0.5	29.3±13.1 (36)	< 0.05
P_2-2MS	0.01	20.9±8.9 (40)	< 0.025
Gl-2MS	0.5	43.0±13.1 (36)	< 0.005
P_4-2MS	0.1	13.5±6.6 (40)	< 0.05
Gl-2MS,S	1.0	37.5±9.4 (63)	< 0.001
Th-3MS	0.1	23.9±6.2 (36)	< 0.001
Pe-4MS	0.5	33.8±5.1 (73)	< 0.001
Th-3MS,S	0.5	36.7±5.5 (35)	< 0.001

[a] Mean values (±S.E.M.) for the increase in insulin output (μU/islet per 90 min) attributable to the esters are derived from comparisons with control data (no ester) from the same experiments. The degrees of freedom are given in parentheses.

3.3. Secretory response to the esters at 16.7 mM D-glucose

The output of insulin from islets incubated in the presence of 16.7 mM D-glucose averaged 233.3 ± 5.5 μU/islet

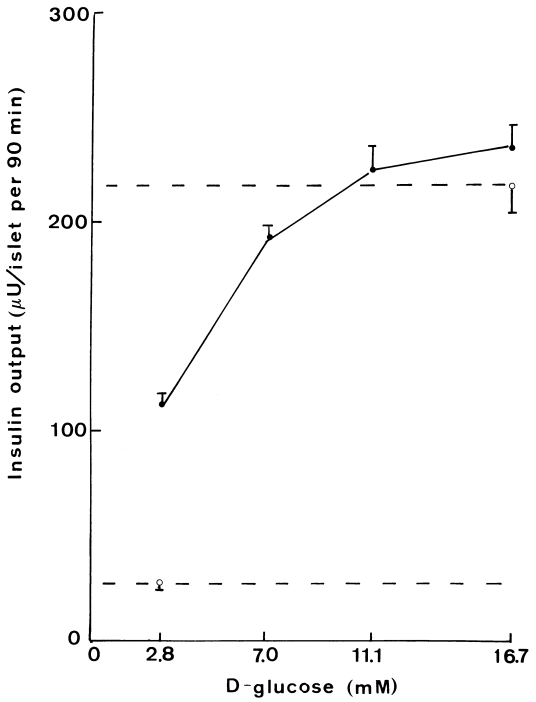

Fig. 6. Insulin release evoked by G-2MS (2.5 mM) at increasing concentrations of D-glucose (closed circles and solid line). The open circles and horizontal dotted lines indicate the values for insulin output recorded within the same experiments at 2.8 and 16.7 mM D-glucose, in the absence of the ester. Mean values (±S.E.M.) refer to 20-41 individual measurements, all collected within the same 4 experiments.

per 90 min ($n = 192$). None of the ten esters examined in the present study increased significantly the insulinotropic action of the hexose (Table 1). On the contrary, three esters, namely G-2ES (1.0 mM) Th-3MS (1.0 mM) and Th-3MS,S (2.0 mM), significantly inhibited ($P < 0.025$ or less) insulin release at the high concentration of the sugar. As documented in Table 1, the same concentration of each given ester was tested in the experiments with either a low (2.8 mM) or a high (16.7 mM) hexose concentration.

Fig. 6 illustrates the hyperbolic pattern of insulin release recorded at increasing concentrations of D-glucose in islets exposed to G-2MS, one of the most potent esters in this series, tested at a concentration (2.5 mM) that elicited a near maximal increase in insulin secretion (see Fig. 2). This pattern differs strikingly from the sigmoidal relationship between insulin output and hexose concentration typically found in islets stimulated with D-glucose alone.

4. Discussion

The present findings confirm that esters of succinic acid display insulinotropic activity (McDonald and Fahien, 1988) and document vast differences in their individual secretory potency.

As mentioned in Section 1, the major aim of this study was to design novel esters with high insulinotropic potency. In this respect, the present work represents the extension of pilot studies in which glycerol-1,2,3-trimethylsuccinate was found to stimulate insulin release either in vitro, when tested at a concentration of only 10 μM (Malaisse et al., 1997), or in vivo, when administered intravenously in an amount not exceeding 0.07 μmol/g body wt. (García-Martínez et al., 1997b).

The comparison between the insulinotropic potency of the esters and their chemical structure calls for the following comments. First, the presence of an unesterified carboxylic group on succinic acid appears to be an unfavourable attribute. This may account, in part at least, for the low potency of TB-1S, although the resistance of tertiary esters to hydrolytic enzymes and the presence of only one succinyl residue in this ester may also play a role in this respect. Likewise, the observation that Th-3MS,S was a less potent secretagogue than Th-3MS (overall insulinotropic scores including the minimal effective concentration: 2.33 ± 0.47 versus 4.16 ± 0.28; $n = 6$ in both cases; $P < 0.01$), despite the presence of 4, rather than 3, succinyl residues in the former ester, could be due to the unesterified acid function of the dicarboxylic acid in position 3 of the threitol backbone. The same situation may also account for the lower insulinotropic potential of Gl-2MS,S compared with Gl-2MS (overall insulinotropic scores 2.01 ± 0.39 versus 3.37 ± 0.32; $n = 7$ in both cases; $P < 0.02$). Moreover, Gl-2MS,S is a less potent secretagogue than glycerol-1,2,3-trimethylsuccinate (Malaisse et al., 1997), as judged *inter alia* from the minimal effective concentration, the two esters differing from one another only by the presence of a free carboxylic function on the succinyl residue in position 3 of the glycerol moiety in Gl-2MS,S.

Second, the presence of an unesterified -OH group on the last C atom in the polyol moiety also seems unfavourable for insulinotropic potency. This is suggested by the low secretory activity of Gl-2MS and P_4-2MS, relative to that of P_2-2MS (overall insulinotropic scores: 3.37 ± 0.32 ($n = 7$) and 3.69 ± 0.57 ($n = 4$) versus 5.70 ± 0.22 ($n = 6$), $P < 0.01$ or less). It is also consistent with the comparable overall insulin scores of P_4-2MS (3.69 ± 0.57; $n = 4$) and Pe-4MS (3.40 ± 0.62; $n = 5$), despite the presence of twice as many methylsuccinyl residues in the latter than in the former ester.

Last, the difference ($P < 0.025$) between the overall insulinotropic scores of G-2MS (4.73 ± 0.49; $n = 6$) and G-2ES (3.06 ± 0.31; $n = 5$) suggests that the esterification of one of the carboxylic functions of succinic acid by methanol, rather than ethanol, favours the secretory potency. This is further supported by comparison of the metabolic and functional responses to the monomethyl and monoethyl esters of succinic acid. Thus, the monomethyl ester, when tested at a 10 mM, significantly augments insulin release from islets incubated either in the absence of D-glucose or at a low concentration (2.8 mM) of the hexose (Malaisse et al., 1993), whilst the monoethyl ester fails to do so (Ladrière et al., 1998). Moreover, when injected intravenously to anaesthetized rats, the monomethyl ester of succinic acid appears, on a molar basis, to be a more potent insulin secretagogue than the monoethyl ester (García-Martínez et al., 1997a).

In islets exposed to 7.0 mM D-glucose, all esters (except Gl-2MS), when tested in high concentrations (2.5 mM or more), lowered insulin output relative to the optimal secretory response found at a lower concentration. In islets exposed to 16.7 mM D-glucose, most esters also decreased mean insulin secretion, this effect being statistically significant in three cases. The mechanism responsible for these untoward effects remains open to speculation. It may be related to a lowering of intracellular pH caused by the hydrolysis of these esters (Jijakli et al., 1996; unpublished observation). This proposal is consistent with the knowledge that islets stimulated by high concentrations of D-glucose are more sensitive to a lowering of extracellular and, hence, intracellular pH than islets incubated in the presence of 7 mM D-glucose. Indeed, whilst a stepwise lowering of extracellular pH below 7.4 causes a progressive inhibition of insulin release from islets exposed to high concentrations of the hexose (11.1 or more), the same environmental change increases insulin output in the 7.4 to 7.0 pH range and only decreases insulin release at lower pH values in islets exposed to 7 mM D-glucose (Hutton et al., 1980).

It could be objected that, in the perspective of developing new insulinotropic tools for the treatment of non-in-

sulin-dependent diabetes, the observation that the present esters failed to enhance insulin secretion from islets incubated at a high concentration of D-glucose represents a rather negative feature. In this disease, however, the B-cell often suffers from a type of 'blindness' to glucose (Malaisse, 1994), with an abnormally low rate of hexose utilization (Malaisse, 1993). The esters of succinic acid may well be expected, therefore, to bypass the metabolic defect(s) responsible for the impaired metabolic and secretory response to glucose. Moreover, such esters, including those examined in the present study (Laghmich et al., 1997), have the advantage, over other insulinotropic antidiabetic agents, that they stimulate both proinsulin biosynthesis and insulin release (Ladrière et al., 1998; Malaisse et al., 1993, 1997).

The design of highly potent insulinotropic esters of succinic acid, such as P_2-2MS, presents two further advantages. First, it counters the objection concerning the poor practicability of daily administration of high doses of the esters to diabetic patients. Second, it avoids the undesirable stimulation of hepatic gluconeogenesis, which could otherwise occur as a result of supplying the esters in such large amounts.

Acknowledgements

This study was supported by a Concerted Action of the French Community of Belgium (94/99-183) and a grant from the Belgian Foundation for Scientific Medical Research (3.4513.94). We are grateful to C. Rémion for technical assistance and to C. Demesmaeker for secretarial help.

References

García-Martínez, J.A., Zhang, T.-M., Villanueva-Peñacarrillo, M.L., Valverde, I., Björkling, F., Malaisse, W.J., 1997a. In vivo stimulation of insulin release by the monoethyl, monopropyl, monoisopropyl, monoallyl and diallyl esters of succinic acid. Res. Commun. Mol. Pathol. Pharmacol. 95, 209–216.

García-Martínez, J.A., Villanueva-Peñacarrillo, M.L., Valverde, I., Björkling, F., Malaisse, W.J., 1997b. Stimulation of insulin release and potentiation of the insulinotropic action of antidiabetic agents by 1,2,3-tri(methylsuccinyl)glycerol ester in anaesthetized rats. Pharmacol. Res. In press.

Hutton, J.C., Sener, A., Herchuelz, A., Valverde, I., Boschero, A.C., Malaisse, W.J., 1980. The stimulus-secretion coupling of glucose-induced insulin release. XLII. Effects of extracellular pH on insulin release: their dependency on nutrient concentration. Horm. Metab. Res. 12, 294–299.

Jijakli, H., Bakkali Nadi, A., Cook, L., Best, L., Sener, A., Malaisse, W.J., 1996. Insulinotropic action of methyl pyruvate. Enzymatic and metabolic aspects. Arch. Biochem. Biophys. 335, 245–257.

Ladrière, L., Louchami, K., Vinambres, C., Kadiata, M.M., Jijakli, H., Villanueva-Penacarrillo, M.L., Malaisse, W.J., 1988. Insulinotropic action of the monoethyl ester of succinic acid. Gen. Pharm., in press.

Laghmich, A., Ladrière, L., Malaisse-Lagae, F., Dannacher, H., Björkling, F., Malaisse, W.J., 1997. Stimulation of biosynthetic activity by novel succinate esters in rat pancreatic islets. Biochem. Pharmacol. In press.

Leclercq-Meyer, V., Malaisse, W.J., 1996. Potentiation of glucagon-like peptide 1 insulinotropic action by succinic acid dimethyl ester. Life Sci. 58, 1195–1199.

Levy, J., Herchuelz, A., Sener, A., Malaisse-Lagae, F., Malaisse, W.J., 1976. Cytochalasin B-induced impairment of glucose metabolism in islets of Langerhans. Endocrinology 98, 429–437.

Malaisse, W.J., 1993. Alteration of pancreatic B-cell D-glucose metabolism in type 2 diabetes: The G quintet. Endocrinologia 40, 309–313.

Malaisse, W.J., 1994. The beta cell in non-insulin-dependent diabetes: Giving light to the blind. Diabetologia 37 (Suppl. 2), S36–S42.

Malaisse, W.J., 1995. The esters of carboxylic nutrients as insulinotropic tools in non-insulin-dependent diabetes mellitus. Gen. Pharm. 26, 1133–1141.

Malaisse, W.J., Rasschaert, J., Villanueva-Peñacarrillo, M.L., Valverde, I., 1993. Respiratory, ionic and functional effects of succinate esters in pancreatic islets. Am. J. Physiol. 264, E428–E433.

Malaisse, W.J., Jijakli, H., Ulusoy, S., Cook, L., Best, L., Viñambres, C., Villanueva-Peñacarrillo, M.L., Valverde, I., Sener, A., 1996. Insulinotropic action of methyl pyruvate: Secretory, cationic and biosynthetic aspects. Arch. Biochem. Biophys. 335, 229–244.

Malaisse, W.J., Ladrière, L., Laghmich, A., Louchami, K., Jijakli, H., Viñambres, C., Villanueva-Peñacarrillo, M.L., Valverde, I., Björkling, F., 1997. Insulinotropic action of 1,2,3-tri(methylsuccinyl)glycerol ester. Biochem. Mol. Med. 62, 76–84.

Malaisse-Lagae, F., Malaisse, W.J., 1984. Insulin release by pancreatic islets. In: Larner, J., Pohl, S.L. (Eds.), Methods in Diabetes Research, Vol. I, part B. John Wiley and Sons, New York, pp. 147–152.

McDonald, M.J., Fahien, L.A., 1988. Glyceraldehyde phosphate and methyl esters of succinic acid. Two "new" potent insulin secretagogues. Diabetes 37, 997–999.

Sener, A., Conget, I., Rasschaert, J., Leclercq-Meyer, V., Villanueva-Peñacarrillo, M.L., Valverde, I., Malaisse, W.J., 1994. Insulinotropic action of glutamic acid dimethyl ester. Am. J. Physiol. 267, E573–E584.

Zhang, T.-M., Sener, A., Malaisse, W.J., 1994. Metabolic effects and fate of succinic acid methyl esters in rat hepatocytes. Arch. Biochem. Biophys. 314, 186–192.