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Stimulation of Insulin and Somatostatin Release by Two Meglitinide Analogs

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Several meglitinide analogs are currently under investigation as potential insulinotropic tools for the treatment of noninsulin-dependent diabetes. The present study aimed to further insight into the effect of these agents on the secretion of insulin, glucagon, and somatostatin by the isolated perfused pancreas. Both repaglinide (0.01 μ*M*) and A-4166 (1.0 μ*M*) stimulated insulin and somatostatin release, but failed to affect glucagon output, from pancreases exposed to 5.6 m*M* D-glucose. The secretory response of the B- and D-cells to the hypoglycemic agents was much less marked than that caused by a rise in hexose concentration from 5.6–16.7 m*M*. Although repaglinide was tested at a concentration a hundred times lower than that of A-4166, the drug-induced increase in both insulin and somatostatin secretion persisted for a longer time after exposure to repaglinide, than to A-4166. The relevance of these findings to the use of meglitinide analogs as antidiabetic agents is double. First, they document that these drugs, although enhancing both insulin and somatostatin release, do not provoke an undesirable stimulation of glucagon secretion. Second, they indicate that even at a very low concentration, repaglinide provokes a protracted insulinotropic action, thus suggesting that the reversibility of the secretory response to this or other meglitinide analogs represents an intrinsic molecular attribute, unrelated to either their biological potency or the relative extent of B-cell stimulation.

Key Words: Meglitinide analogs; insulin secretion; glucagon secretion; somatostatin secretion.

Introduction

Several analogs of meglitinide, the nonsulfonylurea moiety of glibenclamide, such as repaglinide and A-4166, are currently contemplated as new agents for the treatment of noninsulin-dependent diabetes mellitus *(1)*. As is the case for hypoglycemic sulfonylureas, distinct meglitinide analogs differ from one another by their insulinotropic potency, the ED_{50} for stimulation of insulin release by the most potent agent being at least two orders of magnitude lower than that of the weakest compound *(1)*. As also is the case for hypoglycemic sulfonylureas, the meglitinide analogs display variable reversibility of their cationic and secretory effects. For instance, in islets perifused in the absence of D-glucose, KAD-1229 *(2)*, A-4166 *(3)*, and S3075 *(4)*, all provoke a rapidly reversible inhibition of ^{86}Rb outflow from prelabeled islets, whereas the decrease in effluent radioactivity caused by repaglinide persists for at least 20 min after removal of the drug from the perifusate *(4)*, all experiments being conducted at a 10-μ*M* concentration of the antidiabetic agents. Likewise, in islets perifused in the presence of 6.0 m*M* D-glucose, the secretory response to the meglitinide analogs (10 μ*M*) is rapidly reversible in the case of KAD-1229, A-4166, and S3075, but not so with repaglinide *(2–4)*. There is no parallelism, however, between the potency and reversibility of the insulinotropic action of distinct meglitinide analogs (4).

To our knowledge, only scanty information is available on the effect of these hypoglycemic agents on either glucagon or somatostatin release from the endocrine pancreas. In one study, A-4166 was reported to stimulate both insulin and somatostatin release from the isolated rat pancreas *(5)*. Conflicting findings were published, however, concerning the effect of this phenylalanine derivative on glucagon secretion *(5,6)*.

In light of these considerations, the present study aims mainly to investigate two aspects of the islet response to the meglitinide analogs. First, the effect of repaglinide and A-4166 on insulin, glucagon and somatostatin release is examined in the isolated perfused rat pancreas. Second, whether the poor reversibility of repaglinide insulinotropic action can be improved at a low concentration of the drug is explored. The experiments were conducted, therefore, in the presence of only 0.01 μ*M* repaglinide, as distinct from 1.0 μ*M* A-4166.

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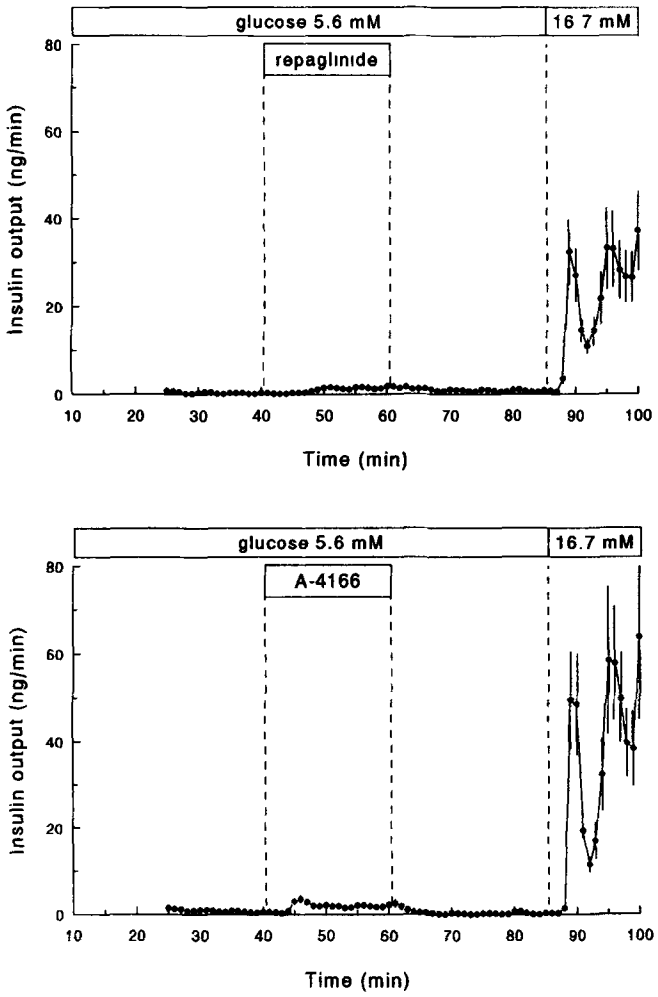


Fig. 1. Effects of repaglinide (0.01 μ*M*, top panel) and A-4166 (1.0 μ*M*, bottom panel) upon insulin release from the rat pancreas perfused in the presence of 5.6 m*M* glucose. The reported values represent the mean (±SE) of four experiments in each case. The drugs were administered for 20 min, as indicated by the vertical dotted lines, no correction being made for the dead space of the perfusion system. A 16.7-m*M* glucose stimulus was applied at the end of all the experiments (vertical dotted line between min 85 and 86).

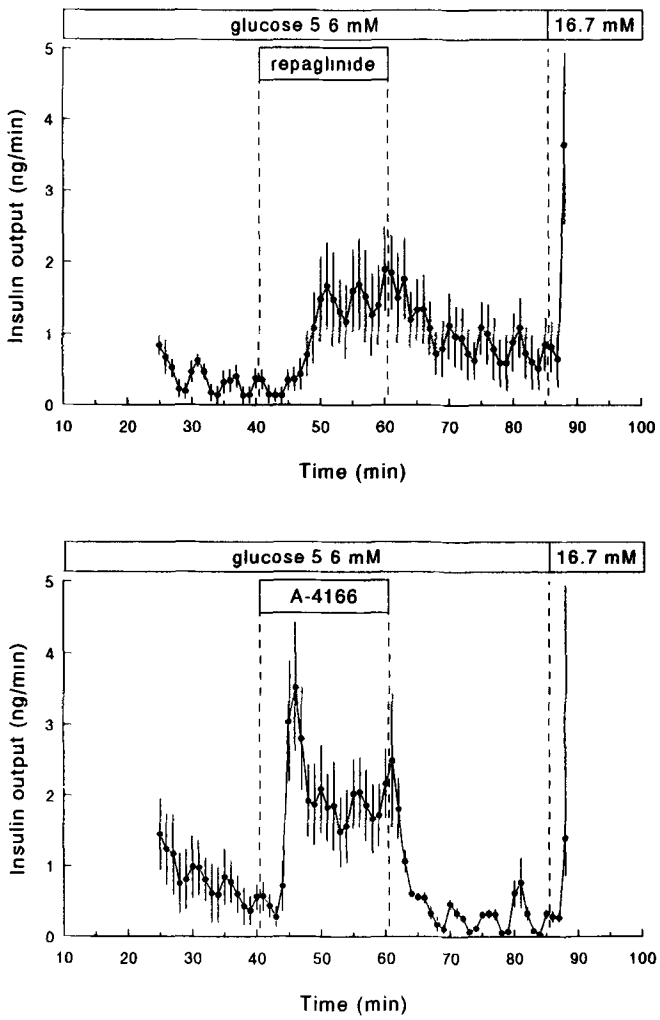


Fig. 2. Effects of repaglinide and A-4166 on insulin release from the perfused rat pancreas. The results are the same as those illustrated in Fig. 1, using an enhanced scale in order to evidence more clearly the respective stimulatory effects of the drugs. The presentation is the same as in Fig. 1.

Results

Insulin Release

In the presence of 5.6 m*M* D-glucose, both repaglinide and A-4166 stimulated insulin release, but to a quite modest extent, when considering the later response of the B-cell to a rise in hexose concentration from 5.6–16.7 m*M* (Fig. 1). The secretory response to A-4166 was rapid with an early secretory peak reached at the 46th min of perfusion. It represented a sustained and rapidly reversible phenomenon (Fig. 2). In the case of repaglinide, which was tested at a hundred times lower concentration than A-4166, a first secretory peak was only reached at the 51st min. When the administration of repaglinide was halted at the 60th min of perfusion, the output of insulin slowly declined towards its initial value. The mean insulin secretory rate during 20 min of exposure of the pancreases to the meglitinide analogs (min 44–63), when expressed relative to the paired value recorded in the presence of 16.7 m*M* D-glucose (min 89–100), averaged 4.4 ± 1.2 and 4.8 ± 0.5% in the case of repaglinide and A-4166. The more rapid reversibility of the secretory response to A-4166 than to repaglinide was documented by the paired ratio between the mean hormonal output after/during exposure to the meglitinide analogs (Table 1). Such a ratio averaged 21.8 ± 7.1% in the case of A-4166, as distinct ($P < 0.02$) from 83.0 ± 15.2% in the case of repaglinide. In all experiments, an oscillatory pattern of insulin release was observed, whether in the absence or presence of the antidiabetic agents and whether at low or high hexose concentration. Such a phasic pattern was even obvious when considering the mean values for insulin release derived, at each time-point, from four individual experiments (Fig. 2). As judged from the latter mean values, the duration of the secretory cycles averaged 5.09 ± 0.17 min ($n = 22$).

Table 1
Paired Ratio (Expressed in %; $n = 4$ in All Cases) for Insulin and Somatostatin Mean Secretory Rates During (Min 44–63) and After (Min 64–88) Exposure to the Meglitinide Analogs or in Response to 16.7 m*M* D-Glucose (Min 89–100)

	Insulin output		Somatostatin output	
Perfusion period (paired ratio)	Repaglinide	A-4166	Repaglinide	A-4166
Min 44–63/min 89–100	4.4 ± 1.2	4.8 ± 0.5	10.9 ± 3.2	24.5 ± 5.1
Min 64–88/min 89–100	3.5 ± 1.2	1.0 ± 0.2	11.9 ± 3.3	12.9 ± 4.0
Min 64–88/min 44–63	83.0 ± 15.2	21.8 ± 7.1	111.3 ± 6.2	49.7 ± 8.5

Table 2
Fractional Release of Pancreatic Hormones at Low and High D-Glucose Concentrations

D-glucose	Insulin	Glucagon	Somatostatin
5.6 m*M* (10^{-6} min^{-1})	4.7 ± 1.4 (8)	26.6 ± 3.7 (8)	27.7 ± 8.0 (8)
16.7 m*M* ($10^{-6} \cdot min^{-1}$)	357.5 ± 87.9 (8)	11.4 ± 1.7 (8)	221.3 ± 58.9 (8)
5.6 m*M*/16.7 m*M* (paired ratio)	75.6 ± 20.2 (8)	0.43 ± 0.04 (8)	6.16 ± 1.87 (8)

Relative to the paired value for the insulin content of the pancreas, the mean output of insulin during the 12 min of exposure to 16.7 mM D-glucose (min 89–100) corresponded to a fractional release of 358 ± 88 10^{-6} min^{-1}, as compared to only 5 ± 2 $10^{-6} \cdot min^{-1}$ during the initial period of perfusion in the sole presence of 5.6 m*M* D-glucose (min 25–43). The paired ratio for insulin output during these two periods was not significantly different in the repaglinide and A-4166 experiments, with an overall mean value of 75.6 ± 20.2 (Table 2).

Glucagon Release

No obvious effect of the meglitinide analogs upon glucagon output could be detected (Fig. 3). The late rise in D-glucose concentration from 5.6–16.7 m*M* resulted, however, in a modest, but sizable decrease in glucagon secretion. Thus, in each experiment, the mean value for glucagon release was lower between the 99th and 100th min than over the preceding 5 min period (min 84–88 inclusive), such a difference achieving statistical significance ($P < 0.02$ or less) except in one individual experiment ($P < 0.07$). The paired ratio between these two values averaged 72.1 ± 2.2% ($n = 8$; $P < 0.001$ as compared to unity). The relative extent of the inhibitory action of D-glucose (16.7 m*M*) upon glucagon release was not significantly different in the two series of experiments dealing with the secretory response to either repaglinide or A-4166.

Somatostatin Release

The changes in somatostatin release during the present experiments resembled, in several respects, those in insulin output. Thus, both repaglinide and A-4166 augmented somatostatin secretion, but to a much lesser extent than observed in response to the late rise in D-glucose concentration (Fig. 4). The stimulation of somatostatin release by

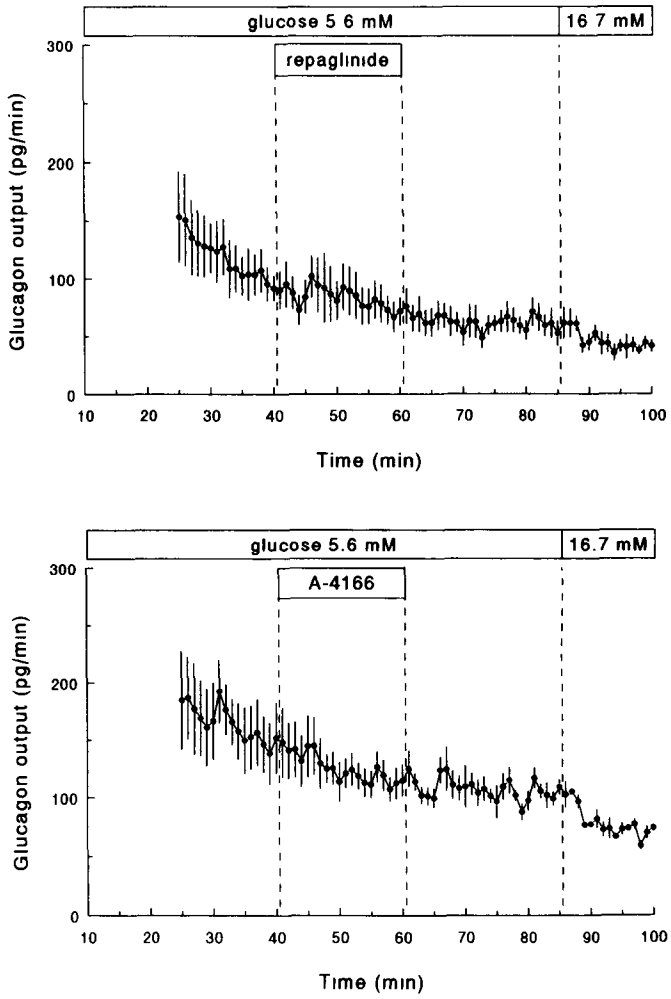
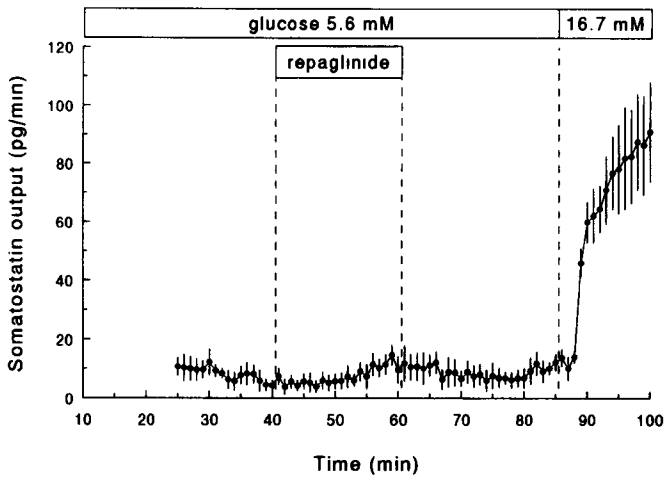


Fig. 3. Effects of repaglinide and A-4166 on glucagon release from the perfused rat pancreas. The presentation is the same as in Fig. 1.

A-4166 was rapid, sustained and rapidly reversible (Fig. 5). A significant increase in secretory rate above the paired



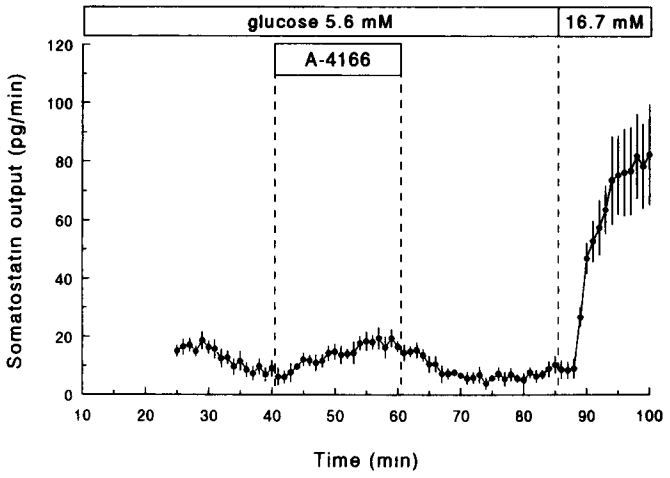
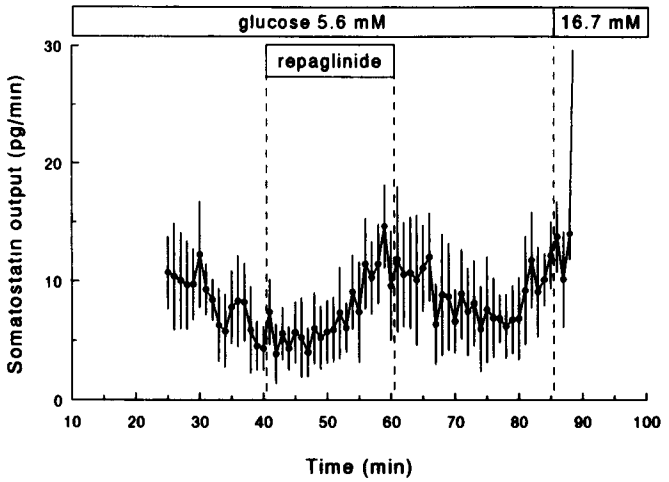


Fig. 4. Effects of repaglinide and A-4166 on somatostatin release from the perfused rat pancreas. The presentation is the same as in Fig. 1.



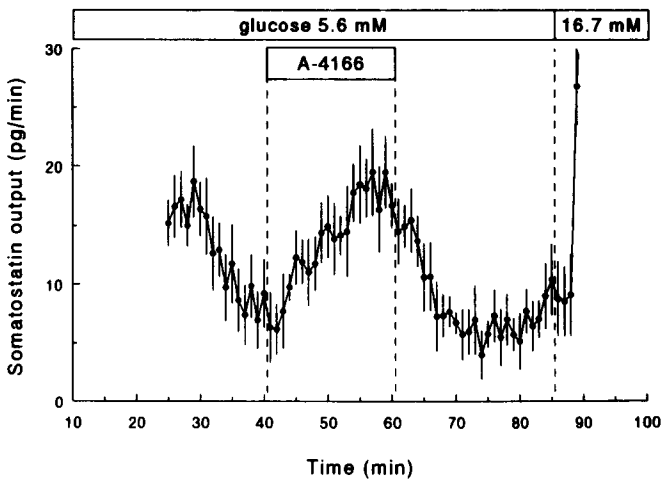


Fig. 5. Effects of repaglinide and A-4166 on somatostatin release from the perfused rat pancreas. The results are the same as those illustrated in Fig. 4, using an enhanced scale in order to evidence more clearly the respective stimulatory effects of the drugs. The presentation is the same as in Fig. 1.

mean control value recorded between the 41st and 43rd min was already detected at the 45th min, it corresponding to an increment of 5.5 ± 0.8 pg/min ($n = 4$; $P < 0.01$). The somatostatin response to repaglinide was more sluggish. A significant increment above the paired control value (min 41–43) was only reached at the 59th min, averaging 9.0 ± 2.5 pg/min ($n = 4$; $P < 0.05$). Even 25 min after cessation of repaglinide delivery, the output of somatostatin remained significantly higher ($P < 0.01$) than the paired control value (min 41–43), with a paired difference of 6.1 ± 1.0 pg/min. Such was not the case in the experiments conducted with A-4166. As already noted in the case of insulin release, the paired ratio in somatostatin output after/during exposure of the pancreas to the antidiabetic agents was lower ($P < 0.005$) in the case of A-4166 than repaglinide (Table 1), confirming the more rapid reversibility of the functional response to the former than the latter agent.

During the first part of the experiments conducted in the presence of 5.6 m*M* D-glucose, the fractional release of somatostatin, expressed relative to the paired hormonal content of the pancreas, averaged $28 \pm 8\ 10^{-6} \cdot \text{min}^{-1}$ (Table 2), which happened to be close to the fractional release of glucagon ($27 \pm 4\ 10^{-6} \cdot \text{min}^{-1}$) at the same time. In contrast with such a situation, the much higher fractional release of somatostatin during stimulation by 16.7 m*M* D-glucose was now comparable ($P > 0.2$) to the insulin output/content ratio found at the same time in these experiments (Table 2). The paired ratio in somatostatin output at 16.7 m*M*/5.6 m*M* D-glucose was not significantly different in the experiments, including administration of either repaglinide or A-4166, with an overall mean value of 6.2 ± 1.9 ($n = 8$).

Perfusion Pressure

None of the agents tested in the present experiments affected the perfusion pressure (data not shown).

Discussion

This study relates to the effect of repaglinide and A-4166 on insulin, glucagon and somatostatin release, the secretory response to these meglitinide analogs being explored

at a concentration of D-glucose close to the threshold value for the insulinotropic action of the hexose and being compared to that evoked by a much higher concentration of the sugar.

As expected, the rise in D-glucose concentration caused a marked increase in insulin and somatostatin output, while inhibiting glucagon secretion. The molar ratio between these hormones, as measured in the pancreas extracts, was in fair agreement with data reported elsewhere *(7)*, the tissue content in glucagon and somatostatin being about 10 and 100 times lower than that of insulin, respectively. Relative to such a content, the secretory rate recorded at the high concentration of D-glucose averaged 3.58 ± 0.88, 0.11 ± 0.02, and 2.21 ± 0.59% in the case of insulin, glucagon, and somatostatin, respectively.

In the present experiments, neither repaglinide nor A-4166 affected significantly glucagon release. In earlier studies also conducted in the perfused rat pancreas at low concentrations of D-glucose (3.0–5.6 m*M*), A-4166 was found either to inhibit *(6)* or stimulate *(5)* glucagon secretion. However, the decrease in glucagon output was observed at a A-4166 concentration of 30 μ*M*, 30 times higher than that used in our experiments, and, in the other study, the authors underlined that A-4166 failed to elicit a clear, dose-related effect on glucagon release. As a matter of fact, over 30 min of exposure to the meglitinide analog, the output of glucagon was much lower at 30 μ*M* A-4166 than at lower concentrations (0.3 and 3.0 μ*M*) of the drug. The present work suggests that at concentrations closer to the therapeutic range, the meglitinide analogs are devoid of any significant glucagonotropic action. This obviously represents a favorable feature in the perspective of their use as antidiabetic agents.

Both hypoglycemic drugs increased insulin, as well as somatostatin release. As little as 0.01 μ*M* of repaglinide was sufficient to evoke such secretory responses. By comparison with a previous report dealing with the effect of A-4166 in the perfused rat pancreas *(5)*, this indicates that the threshold concentration for stimulation of insulin release is lower in the case of repaglinide (<0.01 μ*M*), than A-4166 (approx 0.03 μM). A comparable difference in insulinotropic efficiency was also previously documented in isolated pancreatic islets *(1)*.

The most salient difference between the two meglitinide analogs concerned the reversibility of their effects on either insulin or somatostatin release. Thus, although repaglinide was tested at a concentration a hundred times lower than that of A-4166, the secretory action of the former drug on both B- and D-cells failed to be rapidly reversed after halting its administration. On the contrary, the secretion of both insulin and somatostatin rapidly returned toward its initial level when A-4166 was removed from the perfusion medium.

Advantage could be taken from these vastly different secretory pattern in selecting one of these agents for therapeutic purpose, the choice being inspired by the response considered as most appropriate for the treatment of each individual diabetic patient.

Table 3
Metabolic Characteristics of the Rats and Experimental Parameters of the Perfusions Conducted with A-4166 and Repaglinide (n = 8)

Rat weight (g)	233 ± 7
Plasma glucose (m*M*)	5.0 ± 0.1
Plasma insulin (μU/mL)	48 ± 3
Pancreas wet wt (g)	0.839 ± 0.037
Pancreas insulin content (μg)	88.0 ± 8.4
(μg/g)	104.5 ± 9.3
Pancreas glucagon content (μg)	5.0 ± 0.4
(μg/g)	5.9 ± 0.5
Pancreas somatostatin content (μg)	0.32 ± 0.05
(μg/g)	0.38 ± 0.05
Insulin/glucagon ratio (molar)	10.6 ± 1.2
Insulin/somatostatin ratio (molar)	86.0 ± 14.6
Glucagon/somatostatin (molar)	8.0 ± 1.1
Flow rate (mL/min)	1.50 ± 0.02
Perfusion pressure (mmHg) min 25	24.2 ± 1.1
min 100	26.8 ± 1.2

The difference in reversibility of the secretory response to repaglinide and A-4166, as documented in this study, reproduces the results of prior experiments conducted in perifused islets *(3,4)*, in which case the two antidiabetic agents were tested at the same high concentration (10 μ*M*). Therefore, the present findings reinforce the view that there is no tight parallelism between the insulinotropic potency and reversibility of the cationic and secretory responses to distinct meglitinide analogs. In other words, different molecular determinants might be responsible for the relative potency of a given hypoglycemic agent and the reversibility of its biological action.

In conclusion, the present study provides information relevant to the use of meglitinide analogs as antidiabetic agents. It documents that these drugs act selectively on B- and D-cells, as distinct from glucagon-producing cells. The dissociation between the efficiency and reversibility of their insulinotropic action, when considered in the framework of their molecular conformation *(8)*, may also help in the identification of the structural determinants responsible for these two fundamental functional attributes and, hence, in the design of new agents with both optimal potency and suitable time-related secretory pattern.

Materials and Methods

Animals

The present study was conducted on eight fed female Wistar rats (233 ± 7 g, Table 3). A 0.8-mL blood sample was obtained from the tail prior to anaesthesia for the estimation of blood glucose (Medi-Test strips, Machery-Nagel, Düren, F.R.G.) The plasma was stored at –25°C until time of measurement of its glucose and insulin concentrations.

Perfusion Procedure

The rats were anaesthetized with sodium pentobarbital (42 mg/kg, ip) and the pancreas was perfused free from adjacent organs through both the coeliac and superior mesenteric arteries as previously described *(9,10)*.

The perfusion medium contained the following salts (m*M*): 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), bovine serum albumin (5 g/L, fraction V, RIA grade, RIA BSA, Sigma), and glucose (5.6 m*M*). The perfusate was continuously gassed with a mixture of O_2 and CO_2 (95:5), which resulted in a pH of approx 7.4. It was directed to the pancreas using a peristaltic pump (Minipuls 2, Gilson, Villiers-le-Bel, France).

The glucose concentration of the perfusate was increased from 5.6–16.7 m*M* at the 86th min. Repaglinide (final concentration 0.01 μ*M*) or A-4166 (final concentration 1.0 μ*M*) was first dissolved in dimethyl sulfoxide (DMSO) (final concentration 0.01%, v/v) and administered from the 41st–60th min. A comparable amount of DMSO was administered during the periods which did not include repaglinide or A-4166. All the stimuli were given in saline through side-arm syringes working at a flow rate of 0.075 mL/min (Unita I infusion pump, Braun, Melsungen, Germany). The pressure was recorded throughout the perfusions with a blood pressure monitor (Palmer, London, UK).

Samples of the pancreatic effluent were collected from the portal vein at 1-min intervals, from the 20th min onward, in chilled tubes containing 1500 KIE aprotinin (kallikrein inhibitor units, Trasylol, Bayer, Brussels, Belgium) and 1.8 mg EDTA. After perfusion, the pancreas was dissected free from fat and lymph nodes, weighed, and extracted using acidified ethanol *(11)* and a mechanical homogenizer (Talboys Engineering Corporation, Montrose, PA). Both the effluent samples and the pancreas extracts were stored at –25°C until time of assay.

Analytical Procedures

The plasma glucose concentration was estimated by a hexokinase method (Sigma Diagnostics, Sigma). The measurements of glucagon and insulin in the perfusion samples were performed as previously described, using our own antisera *(12,13)*.

The somatostatin radioimmunoassay (RIA) was adapted from methods described previously *(14–17)*. The assay diluent was a phosphate buffer (0.05 *M*, pH 8.0) containing NaCl (0.14 *M*), EDTA (0.032 *M*), NaN3 (3.1 m*M*), and aprotinin (250 KIE/mL). After these additions, the pH was again adjusted to 8.0 with 5 *M* NaOH. It was supplemented with RIA bovine serum albumin (BSA) (5 g/L,) and gelatin (1 g/L, Type B, from bovine skin, Sigma) on the day of the assay. The standard was synthetic cyclic somatostatin-14 (VB 150, MW 1638, Biogenesis, Poole, UK). It was dissolved in 0.1 *M* acetic acid containing 1 g/L RIA BSA and

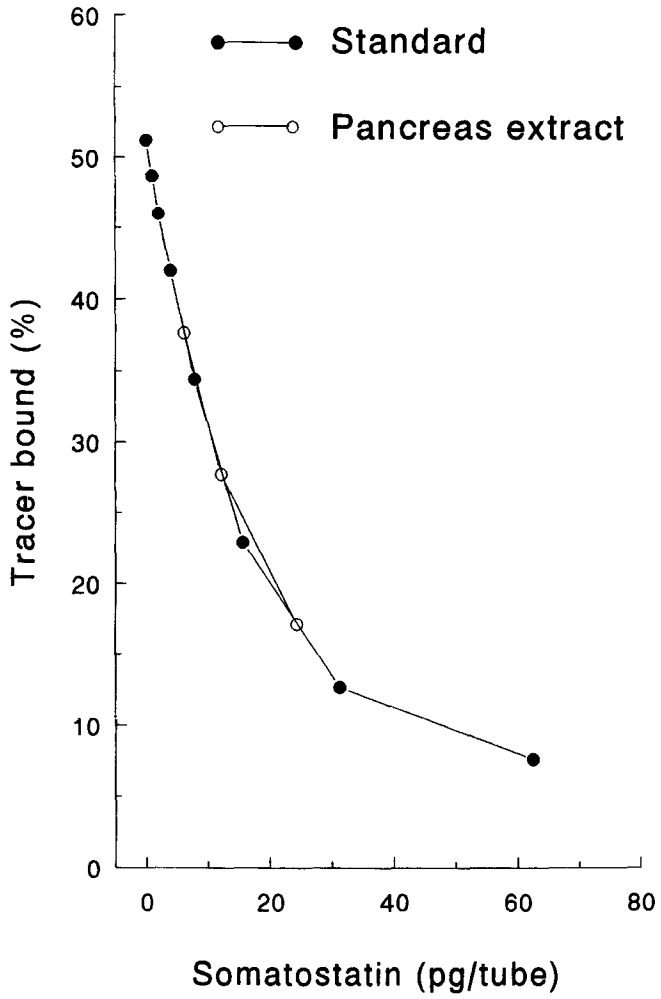


Fig. 6. Representative standard curve obtained with synthetic somatostatin-14 standards and displacement of the tracer bound by the antisomatostatin serum in the presence of a serially diluted extract of the rat pancreas.

stored as a stock solution at –25°C *(16)*. The antisomatostastin serum (L6,3) was raised in rabbit and characterized previously *(13,15)*. It crossreacts about 50% with somatostatin-28 relative to somatostatin-14 on a molar basis, but shows no cross-reaction with other pancreatic hormones, such as glucagon, insulin, or pancreatic polypeptide. The tracer was [^{125}I]Tyr^1-somatostatin (NEX-129, 2200 Ci/mmol, NEN, Dupont de Nemours, Brussels, Belgium). The incubation was conducted in 5-mL plastic tubes in a total volume of 0.6 mL. The reagents were added on the same day in the following order: standards (in triplicate, range 1–62.5 pg in 0.2 mL diluent) or unknown samples (in duplicate, 0.2 mL) a volume of 0.1 mL of diluent, antisomatostatin serum (0.2 mL of diluted antiserum 1/50,000, final dilution 1/150,000) and tracer (4 pg in 0.1 mL). The assay tubes were incubated for 72 h at 4°C, and the separation of free and antibody-bound somatostatin was performed with 1 mL of ice-cold serum-dextran-coated charcoal suspension. The suspension was prepared in phosphate buffer (pH 8.0) and contained bovine serum (100 mL/L,

Serva, Heidelberg, Germany), dextran T70 (2.5 g/L, Pharmacia, Uppsala, Sweden) and neutral charcoal (12.5 g/L, Norit A, Amend Drug and Chemical Co., Irvington, NJ). On further incubation at 4°C for 45 min, the tubes were centrifuged at 4°C for 20 min at 1000*g*. The supernatant was removed by suction, and the radioactivity of the tubes containing the charcoal pellet with the free adsorbed hormone was counted for 4 min (γ-counter, Model 1272, LKB, Uppsala, Sweden).

The sensitivity of the somatostatin RIA as calculated from triplicate standard samples was 0.65 pg/tube. The ID_{80}, i.e., the amount of unlabeled somatostatin that decreased the binding of the tracer to 80% of its reference value (no somatostatin), was 4.2 pg, the ID50 12.8 pg, and the ID20 40.6 pg/tube. As shown in Figure 6, a serially diluted extract of the rat pancreas reacted in parallel with the somatostatin-14 standard.

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.

Acknowledgments

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