

# Glucagon-like peptide-1, but not glucose-dependent insulintropic peptide, inhibits glucagon secretion via somatostatin (receptor subtype 2) in the perfused rat pancreas

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## Abstract

**Aims/hypothesis** The glucose-lowering effect of glucagon-like peptide-1 (GLP-1) is based not only upon its potent insulintropic actions but also on its ability to restrain glucagon secretion. Surprisingly, the closely related glucose-dependent insulintropic peptide (GIP) stimulates glucagon release. We examined whether the islet hormone somatostatin, which strongly inhibits glucagon secretion, is involved in this divergent behaviour.

**Methods** At 1.5 mmol/l glucose and therefore minimal insulin secretion, the glucagon, insulin and somatostatin responses to 20 mmol/l glucose, GLP-1, GIP and somatostatin were studied in the presence of a high-affinity monoclonal somatostatin antibody and of a highly specific somatostatin receptor subtype 2 (SSTR2) antagonist (PRL-2903) in the isolated perfused rat pancreas.

**Results** In control experiments, GLP-1 at 1 and 10 nmol/l reduced glucagon secretion significantly to  $59.0 \pm 6.3\%$  ( $p < 0.004$ ;  $n = 5$ ; SSTR2 series; each vs pre-infusion level) and to  $48.0 \pm 2.6\%$  ( $p < 0.001$ ;  $n = 6$ ; somatostatin antibody series) respectively. During somatostatin antibody administration, GLP-1 still inhibited glucagon secretion significantly, but the effect was less pronounced than in control experiments ( $p < 0.018$ ). Co-infusion of the SSTR2 antagonist completely abolished the GLP-1-induced suppression of glucagon

secretion. In contrast, neither the GIP-induced stimulation of glucagon release nor its inhibition by 20 mmol/l glucose was altered by somatostatin antibody or SSTR2 antagonist administration.

**Conclusions/interpretation** We conclude that GLP-1 is capable of inhibiting glucagon secretion even in the absence of secretory products from the beta cell. It is highly likely that this is mediated via somatostatin interacting with SSTR2 on rat alpha cells. In contrast, GIP and glucose seem to influence the alpha cell independently of somatostatin secretion.

**Keywords** GIP · GLP-1 · Glucagon · Gut hormones · Insulin · Paracrine · Perfused rat pancreas · Somatostatin

## Abbreviations

GIP glucose-dependent insulintropic peptide  
GLP glucagon-like peptide  
SSTR2 somatostatin receptor subtype 2

## Introduction

Both incretin hormones, glucagon-like peptide 1 (GLP-1) and glucose-dependent insulintropic peptide (GIP), are very potent insulin-releasing agents [1]. However, whereas the glucose-lowering action of GLP-1 is amplified by its strong inhibition of glucagon secretion, rendering it particularly attractive for the treatment of type 2 diabetes mellitus [2], GIP has been reported to stimulate glucagon release at basal glucose concentrations in several species, including humans [3, 4]. Given the role of hyperglucagonaemia in the development and progression of type 2 diabetes mellitus [5], it appears worthwhile to study further the effect of the two incretin hormones on glucagon secretion.

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To date, it is unknown whether GLP-1 suppresses glucagon release directly by binding to GLP-1 receptors expressed on the alpha cell, or indirectly by modulating the release of secretory products from the beta or delta cell, such as insulin [6], zinc [7],  $\gamma$ -aminobutyric acid [8] or somatostatin [9]. Reports on the expression of GLP-1 receptors on alpha cells have been inconsistent, either failing to detect GLP-1 receptors at all [10, 11] or identifying them only in a subdivision of alpha cells [12]. In contrast, it is well known that GLP-1 stimulates insulin and somatostatin secretion via GLP-1 receptors expressed on beta and delta cells [13, 14]. Somatostatin is a potent suppressor of insulin and glucagon release [15, 16] and has been suggested to act as an intra-islet regulator of glucagon secretion in rats [9]. This concept, however, has been strongly argued against by Samols and Stagner, who, based on immunoneutralisation studies in the isolated perfused pancreas of dogs, rats and humans, postulated that the order of microvascular cellular perfusion in pancreatic islets is from beta to alpha to delta cells, ruling out a substantial regulatory role for somatostatin [17–19].

Somatostatin exerts its numerous biological actions via five distinct receptor subtypes (SSTR1–5), all of which have been shown to be expressed in human and rat pancreatic islets [20–22]. However, SSTR2 has been identified as the key mediator of somatostatin-induced inhibition of glucagon release in rats using SSTR2-selective agonists and antagonists, as well as SSTR2 knockout mice [9, 23–25].

Hence, the aim of this study was to evaluate the involvement of somatostatin and SSTR2 in the GLP-1-induced inhibition of glucagon release using immunoneutralisation with high-affinity monoclonal somatostatin antibodies [26], which should block the actions of somatostatin regardless of the target receptor subtype, as well as the highly specific SSTR2 antagonist PRL-2903, also known as DC-41-33 [27]. In addition, we wished to investigate how blocking of delta cell action might influence the effect of GIP and glucose on glucagon secretion, given the fact that GIP and glucose, like GLP-1, have been reported to stimulate pancreatic somatostatin secretion [28–32]. As a model, we used the isolated perfused rat pancreas, in which the integrity of the islet's cytoarchitecture and microvasculature is preserved, which is crucial when investigating paracrine interactions between islet cells. Moreover, to minimise the influence of secretory products from the beta cell, experiments were performed at a glucose concentration of 1.5 mmol/l.

## Methods

This study conformed to the Danish legislation governing animal experimentation, and was carried out after permis-

sion had been granted from the National Superintendence for Experimental Animals.

**Animals** Female Wistar rats (220–350 g, 10–12 weeks of age) were purchased from Charles River, Sulzfeld, Germany more than 1 week before the experiments were performed, and given free access to standard rodent chow and water. Animals were housed two per cage and were subjected to a 12:12 h light–dark cycle.

**Isolated perfused rat pancreas** Non-fasted rats were anaesthetised with an i.p. injection of pentobarbital (50 mg/kg; Royal Veterinary and Agriculture University, Frederiksberg, Denmark), and the pancreas was dissected and perfused in situ as described [33]. Briefly, the rat was killed by removal of the heart, and the pancreas perfused in a single-pass system through both the coeliac and the superior mesenteric artery via a catheter inserted into the adjacent abdominal aorta. All other aortic branches were ligated. The venous effluent was collected for 1 min intervals via an obstructing cannula inserted into the portal vein, and stored at  $-20^{\circ}\text{C}$  until analysis. The flow rate was kept constant at 4 ml/min. The perfusion medium was continuously gassed with a 95%  $\text{O}_2$ /5%  $\text{CO}_2$  mixture to achieve pH 7.4, and maintained at  $37^{\circ}\text{C}$  during the entire experiment.

**Somatostatin antibody studies** In somatostatin antibody experiments ( $n=12$ ), the perfusion medium consisted of a modified Krebs–Ringer–bicarbonate buffer, containing in addition 5% human serum albumin (Behringwerke, Marburg, Germany), 1.5 mmol/l glucose and 5 mmol/l each of pyruvate, fumarate and glutamate. High-affinity (affinity constant  $10^{11}$  l/mol) monoclonal mouse anti-somatostatin IgG1 antibodies (SOM-18; kindly provided by Novo Nordisk, Bagsværd, Denmark) were dialysed against saline for at least 20 h before use and prepared to give an antibody solution yielding a final somatostatin binding capacity of 6.7 nmol/ml in the perfusate [26]. To raise the glucose concentration in the perfusate temporarily from 1.5 to 20 mmol/l, a concentrated glucose solution (500 g/l; SAD, Copenhagen, Denmark) was administered via a sidearm syringe. Human GIP (Bachem, Bubendorf, Switzerland), synthetic GLP-1 (7–37) (a gift from Novo Nordisk, Bagsværd, Denmark) and somatostatin (Bachem, Bubendorf, Switzerland) were dissolved in 0.9% NaCl containing 1% human serum albumin. Final perfusate concentrations were 20 mmol/l (glucose) and 10 nmol/l (GIP, GLP-1, somatostatin).

After a 30 min equilibration period, a continuous infusion of somatostatin antibody (minutes 0–160) was initiated ( $n=6$ ), whereas in control experiments no somatostatin antibody was administered ( $n=6$ ). After perfusion with basal medium for 15 min (minutes 0–15), glucose (final concentration 20 mmol/l), GIP (10 nmol/l), GLP-1 (10 nmol/l) and

somatostatin (10 nmol/l) were infused for 15 min each (minutes 16–30, 56–70, 96–110 and 136–150, respectively) separated by 25 min rest periods, during which hormone secretion returned to basal levels.

**Somatostatin receptor subtype 2 antagonist studies** In SSTR2 antagonist experiments ( $n=12$ ), the perfusion medium consisted of a modified Krebs–Ringer–bicarbonate buffer, containing, in addition, 0.1% human serum albumin, 5% dextran T-70 (Pharmacia Biotech, Uppsala, Sweden), 1.5 mmol/l glucose and 5 mmol/l each of pyruvate, fumarate and glutamate. The SSTR2 antagonist PRL-2903, also known as DC-41-33 (Peptide Research Laboratories, Tulane University School of Medicine, New Orleans, LA, USA) shows both high selectivity and potency for human SSTR2, with a binding affinity of  $26.4 \pm 3.1$  nmol/l and an  $IC_{50}$  of  $2.5 \pm 0.6$  nmol/l against 1 nmol/l somatostatin in a rat in vitro antagonist bioassay [27, 34]. PRL-2903, GLP-1 (7-37), GIP and somatostatin were dissolved in 0.9% NaCl containing 1% human serum albumin and infused into the arterial line using a syringe pump to give final perfusate concentrations of 100 nmol/l (SSTR2 antagonist) or 1 nmol/l (GLP-1, GIP, somatostatin). A concentrated glucose solution (500 g/l) was administered via a sidearm syringe to raise the glucose concentration in the perfusate temporarily from 1.5 to 20 mmol/l.

After a 30 min equilibration period, sampling of the effluent was started and the pancreas was perfused with basal perfusion medium for 15 min (minutes 0–15). In a first set of experiments ( $n=5$ ), 1 nmol/l GLP-1 and 1 nmol/l somatostatin were each infused for 10 min (minutes 16–25 and 41–50, respectively), followed by 15 min rest periods to allow endocrine secretion to return to basal levels. From minute 66 to 130, 100 nmol/l SSTR2 antagonist was infused continuously. Twenty minutes after the start of the SSTR2 antagonist infusion, 1 nmol/l GLP-1 and 1 nmol/l somatostatin were each infused for 10 min (minutes 86–95 and 111–120, respectively) separated by a 15 min rest period. In a second set of experiments ( $n=7$ ) the same protocol was followed, except that 20 mmol/l glucose and 1 nmol/l GIP instead of GLP-1 and somatostatin were infused.

**Hormone analysis** Pancreatic insulin, glucagon and somatostatin concentrations in venous effluent were analysed by RIA. Insulin concentrations were determined using guinea-pig antiserum raised against porcine insulin (2006-3), which cross-reacts strongly with both rat insulin I and II, and human  $^{125}$ I-labelled insulin labelled in position A14. A mixture (2:1) of rat insulin I and II was used as standard (Novo Nordisk) [35]. Glucagon immunoreactivity was measured by means of the C-terminally directed antiserum 4305, which only detects glucagon of pancreatic origin, and highly purified porcine glucagon as standard [36]. Somato-

statin immunoreactivity was determined using antiserum 1758, which was raised in rabbits against synthetic cyclic somatostatin and recognises both somatostatin-14 and somatostatin-28 [37, 38].

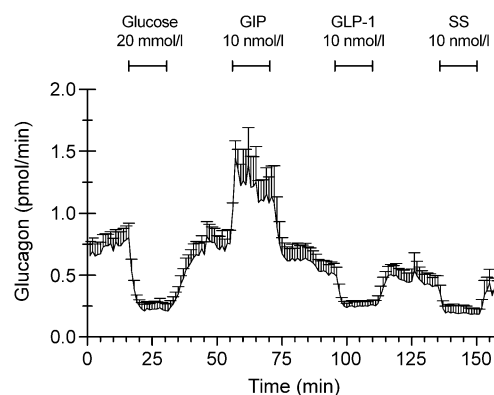
**Calculations and statistical analysis** Results are expressed in pmol/min or fmol/min or as the percentage of the prestimulatory secretion (mean  $\pm$  SEM for indicated numbers of experiments). For each stimulus, the mean hormone output during the immediate prestimulatory 5 min period was defined as 100% and compared with the mean hormone output during which maximal hormone response was observed (for stimulatory agents, minutes 3–7 after initiation of the infusion; for inhibitory agents, the last 5 min of the infusion). The statistical significance of changes was evaluated by the two-tailed  $t$  test for paired or unpaired data, with  $p < 0.050$  being considered significant, using Statistica, version 7 (StatSoft, Tulsa, OK, USA).

## Results

**Somatostatin antibody studies** The basal glucagon secretion in control experiments did not differ significantly from the basal glucagon secretion observed in somatostatin antibody experiments ( $0.718 \pm 0.11$  pmol/min without somatostatin antibody vs  $0.637 \pm 0.15$  with somatostatin antibody).

In control experiments ( $n=6$ ), glucagon secretion was potently inhibited by 20 mmol/l glucose (to  $25.7 \pm 3.5\%$  of prestimulatory secretion;  $p < 0.001$ ). Somatostatin at 10 nmol/l suppressed glucagon release to  $37.4 \pm 4.3\%$  ( $p < 0.001$ ) of the basal value (Fig. 1). GLP-1 and GIP at 10 nmol/l showed completely opposite effects, GLP-1 strongly suppressing glucagon secretion to  $48.0 \pm 2.6\%$  ( $p < 0.001$ ) and GIP stimulating it to  $196.2 \pm 30.0\%$  ( $p < 0.024$ ) of basal values.

When the monoclonal somatostatin antibody was constantly co-infused, GLP-1 still inhibited glucagon release



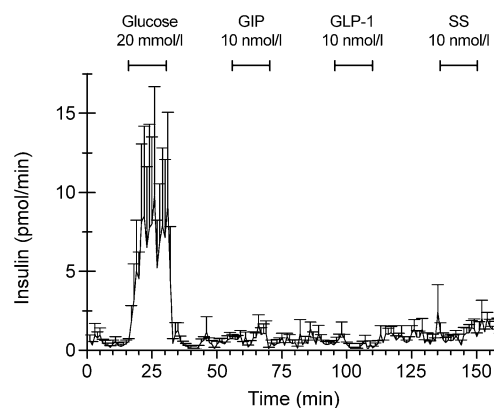
**Fig. 1** Glucagon secretion from the perfused rat pancreas in somatostatin (SS) antibody control experiments during perfusion with 1.5 mmol/l glucose ( $n=6$ ). Data are mean  $\pm$  SEM

(to  $61.7 \pm 4.9\%$ ;  $p < 0.001$ ;  $n = 6$ ) (Fig. 2); however, this inhibition was significantly attenuated compared with control experiments ( $61.7 \pm 4.9\%$  with vs  $48.0 \pm 2.6\%$  without somatostatin antibody;  $p < 0.018$ ). Similarly, somatostatin continued to suppress glucagon secretion in the presence of somatostatin antibody ( $p < 0.007$ ), but this effect was much less pronounced than in the absence of somatostatin antibody (to  $65.3 \pm 7.9\%$  with vs  $37.4 \pm 4.3\%$  without somatostatin antibody;  $p < 0.011$ ).

In contrast, neither the inhibitory effect of 20 mmol/l glucose (to  $35.5 \pm 5.6\%$  of basal secretion with somatostatin antibody vs  $25.7 \pm 3.5\%$  without,  $p = \text{NS}$ ) nor the stimulatory effect of GIP ( $183.4 \pm 32.1\%$  with somatostatin antibody vs  $196.2 \pm 30.0\%$  without;  $p = \text{NS}$ ) on glucagon secretion were altered by co-administration of somatostatin antibody.

As expected, only infusion of 20 mmol/l glucose induced a strong increase in insulin secretion, whereas the insulinotropic effect of GLP-1 and GIP was completely abolished at 1.5 mmol/l glucose in both somatostatin antibody and corresponding control experiments (Fig. 3). No effect of somatostatin on insulin output was observed at this low rate of secretion. In control experiments, somatostatin release was increased by both 20 mmol/l glucose and 10 nmol/l GLP-1 and GIP (Fig. 4), whereas no somatostatin was detectable in somatostatin antibody experiments (data not shown).

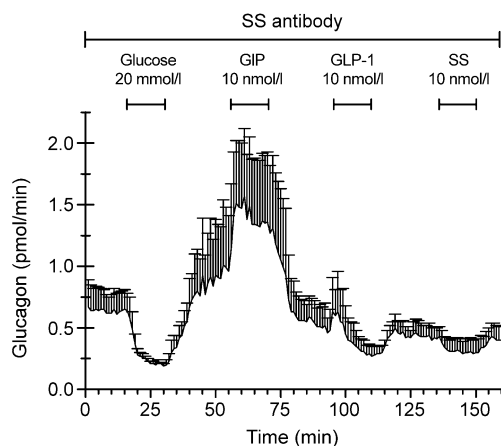
**Somatostatin receptor subtype 2 antagonist studies** In a second series of experiments ( $n = 5$ ), we applied GLP-1 and somatostatin, each at 1 nmol/l, in the presence and absence of the highly specific SSTR2 antagonist PRL-2903. The basal glucagon secretion was  $0.526 \pm 0.08$  pmol/min. Infusion of the antagonist itself resulted in an abrupt and steep rise in glucagon secretion to  $234.1 \pm 9.7\%$  of the pre-infusion level ( $p < 0.001$ ) (Fig. 5). GLP-1 alone reduced glucagon



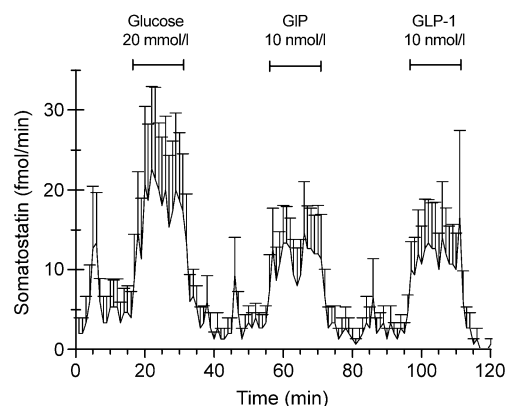
**Fig. 3** Insulin secretion from the perfused rat pancreas in somatostatin (SS) antibody control experiments during perfusion with 1.5 mmol/l glucose ( $n = 6$ ). Data are mean  $\pm$  SEM

release to  $59.0 \pm 6.3\%$  of the prestimulatory level ( $p < 0.003$ ), whereas somatostatin suppressed it to  $51.0 \pm 15.2\%$  ( $p < 0.032$ ). However, when administered during infusion with the SSTR2 antagonist, the inhibitory effect of both agents was completely abolished, glucagon secretion now being  $92.2 \pm 8.5\%$  (GLP-1) and  $98.2 \pm 7.8\%$  (somatostatin) of pre-infusion levels ( $p = \text{NS}$ ). These hormone responses differed significantly from those observed in the absence of the SSTR2 antagonist ( $p < 0.004$  for GLP-1;  $p < 0.029$  for somatostatin) (Fig. 6).

To examine the effect of the SSTR2 antagonist on GIP- and glucose-regulated glucagon release, we completed a third set of experiments in which 20 mmol/l glucose and 1 nmol/l GIP were given ( $n = 7$ ). The basal glucagon secretion was  $0.804 \pm 0.16$  pmol/min. In agreement with the experiments with GLP-1 and somatostatin, administration of the SSTR2 antagonist induced a prompt and pronounced increase in glucagon secretion to  $322.2 \pm 48.8\%$  of the pre-infusion level ( $p < 0.004$ ) (Fig. 7). Infusion

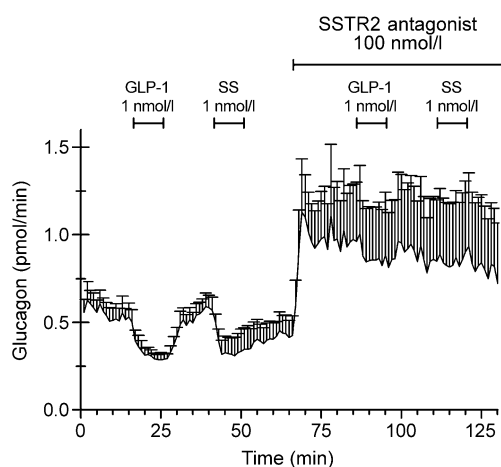


**Fig. 2** Glucagon secretion from the perfused rat pancreas in somatostatin (SS) antibody experiments during perfusion with 1.5 mmol/l glucose ( $n = 6$ ). Data are mean  $\pm$  SEM



**Fig. 4** Somatostatin secretion from the perfused rat pancreas in somatostatin (SS) antibody control experiments during perfusion with 1.5 mmol/l glucose ( $n = 6$ ). Data are mean  $\pm$  SEM





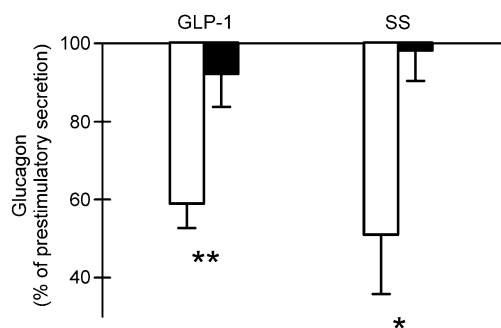
**Fig. 5** Glucagon secretion from the perfused rat pancreas in SSTR2 antagonist experiments, in which 1 nmol/l GLP-1 and somatostatin were infused, during perfusion with 1.5 mmol/l glucose ( $n=5$ ). Data are mean $\pm$ SEM

of 20 mmol/l glucose inhibited glucagon secretion to  $29.7 \pm 7.0\%$  of the prestimulatory level ( $p < 0.001$ ), whereas GIP stimulated it up to  $186.6 \pm 18.6\%$  ( $p < 0.004$ ). During co-infusion of the SSTR2 antagonist, glucagon secretion was still restrained by 20 mmol/l glucose ( $44.1 \pm 10.5\%$  of the pre-infusion level;  $p < 0.002$ ) and still increased by GIP (to  $174.5 \pm 36.5\%$ ;  $p = 0.08$ ), and these hormone responses did not differ significantly from those measured in the absence of the antagonist.

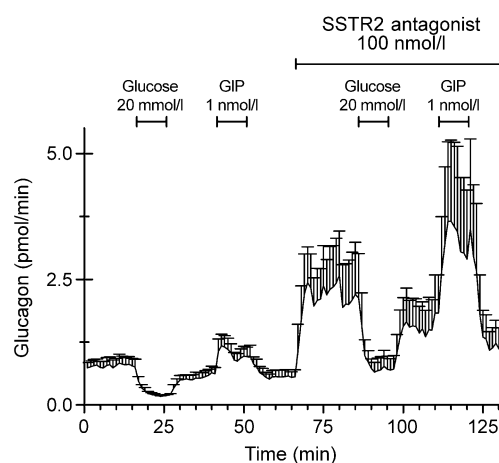
Somatostatin release was significantly increased by both GLP-1 and GIP to  $211.2 \pm 33.2$  and  $180.7 \pm 16.9\%$ , respectively (data not shown).

## Discussion

GLP-1 and GIP display their main glucoregulatory effects on the endocrine part of the pancreas, where they exert a



**Fig. 6** Glucagon output expressed as percentage of prestimulatory secretion in response to 1 nmol/l GLP-1 and 1 nmol/l somatostatin (SS) without infusion of SSTR2-antagonist (white bars) compared with glucagon output during infusion of 100 nmol/l SSTR2-antagonist (black bars);  $n=5$ . For each stimulus, the mean hormone output during the prestimulatory 5 min period was defined as 100%. Data are mean $\pm$ SEM. \*  $p < 0.05$ ; \*\*  $p < 0.005$



**Fig. 7** Glucagon secretion from the perfused rat pancreas in SSTR2-antagonist experiments, in which 20 mmol/l glucose and 1 nmol/l GIP were infused, during perfusion with 1.5 mmol/l glucose ( $n=7$ ). Data are mean $\pm$ SEM

potent insulinotropic influence on the beta cell. Because GLP-1 and GIP also modulate the secretion of the alpha and delta cell, it is tempting to speculate that the regulation of glucose homeostasis by incretin hormones is the result of their combined actions on all three islet cells. Interestingly, whereas GLP-1 and GIP both stimulate insulin secretion, they show opposite effects on glucagon secretion [4, 13]. At present, the underlying mechanism of this differential behaviour has not been elucidated and studies of the expression of GLP-1 receptor on alpha cells have produced conflicting results [10–12].

Hence, in this study we examined the involvement of somatostatin in the GLP-1- and GIP-mediated regulation of glucagon secretion in the isolated perfused rat pancreas using different tools to antagonise somatostatin action. Both the monoclonal somatostatin antibody and the SSTR2 antagonist have been employed in previous studies to examine the role of somatostatin as a mediator of the inhibitory actions of gut hormones on gastric acid release [26, 34, 39].

The most important finding in our study was that infusion of the highly selective SSTR2 antagonist PRL-2903 (DC-41-33) completely prevented the GLP-1-induced inhibition of glucagon secretion, whereas only partial attenuation was observed during infusion of monoclonal somatostatin antibody. We observed also an immediate and rapid rise in basal glucagon secretion in response to the administration of the SSTR2 antagonist, indicating that the alpha cell is under the tonic control of somatostatin. These findings provide strong support for the idea of an indirect inhibitory action of GLP-1 on glucagon secretion, which is mediated by somatostatin acting on SSTR2. In addition, they underline the essential role of somatostatin as an intra-islet regulator of glucagon secretion, and are in line with

recent studies in isolated islets from SSTR2 knockout mice [24], the isolated perfused rat pancreas [9] and in isolated human islets [25].

In our study, the antagonism of GLP-1's glucagonostatic effect differed considerably between the two experimental series, the SSTR2 antagonist completely and the monoclonal somatostatin antibody only partially reversing the inhibition of glucagon release. We assume that the results of the antibody experiment are unlikely to reflect incomplete immunoneutralisation by the antibody employed, since previous studies from our laboratory have shown a high degree of effectiveness at the antibody concentration chosen [26]. Instead, the reason for this discrepancy might be the structural difference between the antibody and the receptor antagonist, which, with a molecular weight of 1,160, is more than 100-fold smaller than the IgG with an approximate molecular weight of 150,000 [27, 40]. Consequently, the SSTR2 antagonist PRL-2903 might be able to access the tight intercellular space between alpha and delta cells without any restrictions, whereas the antibody is prevented from reaching this site of paracrine action because of steric limitations. In support of this assumption, it has been demonstrated that the capillaries of the endocrine pancreas, despite being highly permeable to nutrients and endocrine hormones, show restricted permeability to Ig [41, 42].

Indeed, the theoretical possibility exists that the antagonist itself might exhibit a direct stimulatory effect on the alpha cell and thus contribute to the results observed. However, in view of the complete lack of agonist activity of the whole family of SSTR antagonists to which PRL-2903 belongs, this possibility seems very unlikely.

Our results appear inconsistent with reports by Samols et al. [17] and Samols and Stagner [18], who perfused isolated rat pancreases both anterogradely and retrogradely with antibodies directed against insulin and somatostatin. Neither insulin nor glucagon secretion increased in response to anterograde perfusion with anti-somatostatin gamma-globulin in their experiments, but instead immediately after reversal of the flow. Hence, the authors hypothesised that islet blood flow was directed from the beta to the alpha to the delta cell and that somatostatin secreted from the downstream located delta cell could not regulate the upstream-located beta and alpha cells under physiological conditions. Considering our findings, the study of Stagner and Samols probably does not reflect the substantial contribution of paracrine interactions to the regulation of intra-islet hormone secretion, as somatostatin gamma-globulin instead of a smaller somatostatin receptor antagonist was applied.

Brunnicardi et al. [43] reported increased basal glucagon secretion during somatostatin antibody administration in the isolated perfused human pancreas. Perhaps differences with

respect to the concentration and type of antibody applied, but also differences between species regarding the permeability of the pancreatic capillaries to Ig, can explain why we could not observe this effect in our study.

Recently, a role for glutamate as a positive autocrine signal for glucagon release has been proposed [44]. Considering that the perfusion medium used in this study contained glutamine to ameliorate respiration, we cannot exclude the possibility that part of the glutamine may have been metabolised to glutamate by the enzyme glutaminase and thereby may have influenced the preparation. However, as the proportion of glutamine was kept constant in all experiments, a possible effect on the alpha cell will have affected all experiments identically, i.e. both control experiments and experiments in which somatostatin antibody or the SSTR2 antagonist was given.

Several reports have ascribed to insulin, as well as other secretory products of the beta cell, a substantial role in the regulation of glucagon secretion [6–8, 10, 17, 45]. In our study we applied a glucose concentration of 1.5 mmol/l in order to minimise the influence of the beta cell. At this glucose level, basal insulin secretion was very low or undetectable, and neither GLP-1 nor GIP stimulated insulin secretion. The fact that GLP-1 still affected glucagon secretion indicates that release of insulin is not an indispensable precondition for GLP-1-induced suppression of glucagon secretion. However, we cannot rule out the possibility that even miniscule amounts of insulin still have an influence.

Consistent with other reports, GIP significantly stimulated glucagon secretion in our experiments [3, 4]. However, unlike GLP-1-mediated inhibition of glucagon release, the GIP-induced enhancement of glucagon release was not influenced by administration of either the monoclonal somatostatin antibody or the SSTR2 antagonist, suggesting a direct, somatostatin-independent action of GIP on the alpha cell. In support of this notion, GIP has been demonstrated to markedly increase the cyclic AMP content in islet alpha cells isolated by FACS, and mRNA from these cells hybridised with GIP receptor cDNA [11]. However, it has to be considered that cell populations obtained by FACS do not represent pure alpha cell populations, but are contaminated with a certain number of other islet cells. Immunohistochemical studies by our group (C. Orskov, J. de Heer and J. J. Holst, unpublished results) showing colocalisation of GIP receptor and proglucagon in human pancreatic islets are consistent with these results.

Interestingly, despite having a stimulatory effect on glucagon secretion, 1 and 10 nmol/l GIP, like GLP-1, stimulated the release of somatostatin. This finding is in line with other studies describing an effect of GIP on somatostatin release [28–30]. However, the lack of effect of somatostatin antibody and SSTR2 antagonist administration on the GIP-induced stimulation of glucagon secretion (one

would expect an increase) suggests that the somatostatin released had no influence on the alpha cells. To date, it is unknown whether GIP receptors are expressed in delta cells. Thus, the observed rise in somatostatin secretion could either be caused directly by GIP or indirectly by enhanced levels of glucagon, which have been demonstrated to stimulate somatostatin release, thus representing a possible negative feedback mechanism [46]. At any rate, the direct glucagonotropic effect of GIP clearly outweighs any paracrine inhibitory effect of somatostatin on glucagon secretion.

Likewise, although 20 mmol/l glucose strongly elicited somatostatin release, the inhibitory effect of glucose on glucagon secretion was not altered by blocking somatostatin action (one would have expected attenuation). This suggests that glucose-induced inhibition of glucagon secretion is independent of delta cell action, which is in agreement with other reports also providing strong evidence for a direct effect of glucose on the alpha cell [47–49], among these a recent one showing that glucose directly inhibits mouse alpha cells by suppression of store-operated depolarising  $\text{Ca}^{2+}$  currents [50].

In conclusion, we demonstrated that GLP-1 inhibits glucagon secretion in the perfused rat pancreas by stimulation of the release of intra-islet somatostatin binding to SSTR2. This inhibition occurs independently of secretory products from the beta cell. In addition, basal glucagon secretion appears to be under the tonic control of somatostatin interacting with SSTR2. In contrast, GIP potently stimulates glucagon secretion equally with or without somatostatin immunoneutralisation or SSTR2 antagonism, indicating a possible direct stimulatory effect on the alpha cell. Our results suggest also that immunoneutralisation, even with high-affinity antibodies, is insufficient to interfere with paracrine mechanisms.

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**Duality of interest** The authors declare that there is no duality of interest associated with this manuscript.

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