Proghrelin-derived peptides influence the secretion of insulin, glucagon, pancreatic polypeptide and somatostatin: A study on isolated islets from mouse and rat pancreas

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Short title: Proghrelin-derived peptides and islet hormone secretion

Abbreviations: PP, pancreatic polypeptide; RIA, radioimmunoassay

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Abstract

Proghrelin, the precursor of the orexigenic and adipogenic peptide hormone ghrelin, is synthetized in endocrine (A-like) cells in the gastric mucosa. During its cellular processing, proghrelin gives rise to the 28-amino acid peptide desacyl ghrelin, which after octanoylation becomes active acyl ghrelin, and to the 23-amino acid peptide obestatin, claimed to be a physiological opponent of acyl ghrelin. This study examines the effects of the proghrelin products, alone and in combinations, on the secretion of insulin, glucagon, pancreatic polypeptide (PP) and somatostatin from isolated islets of mice and rats. Surprisingly, acyl ghrelin and obestatin had almost identical effects in that they stimulated the secretion of glucagon and inhibited that of PP and somatostatin from both mouse and rat islets. Obestatin inhibited insulin secretion more effectively than acyl ghrelin. In mouse islets, acyl ghrelin inhibited insulin secretion at low doses and stimulated at high. In rat islets, acyl ghrelin inhibited insulin secretion in a dose-dependent manner but the IC_{50} for the acyl ghrelin-induced inhibition of insulin release was $7.5x10^{-8}$ M, while the EC_{50} and IC_{50} values, with respect to stimulation of glucagon release and to inhibition of PP and somatostatin release, were in the $3x10^{-12}$-$15x10^{-12}$ M range. The corresponding EC_{50} and IC_{50} values for obestatin ranged from $5x10^{-12}$ to $20x10^{-12}$ M. Desacyl ghrelin *per se* did not affect islet hormone secretion. However, at a ten times higher concentration than acyl ghrelin (corresponding to the ratio of the two peptides in circulation), desacyl ghrelin abolished the effects of acyl ghrelin but not those of obestatin. Acyl ghrelin and obestatin affected the secretion of glucagon, PP and somatostatin at physiologically relevant concentrations; with obestatin this was the case also for insulin secretion. The combination of obestatin, acyl ghrelin and desacyl ghrelin in concentrations and proportions similar to those found in plasma resulted in effects that were indistinguishable from those induced by obestatin alone. From the

data it seems that the effects of endogenous, circulating acyl ghrelin may be overshadowed by obestatin or blunted by desacyl ghrelin.

Keywords: Ghrelin; Acyl ghrelin; Desacyl ghrelin; Obestatin; Proghrelin; Pancreatic Hormones; Pancreatic Islets; Insulin; Glucagon; Pancreatic Polypeptide; Somatostatin

1. Introduction

Ghrelin is a gastric hormone [1] that affects energy balance [2-5] and gastrointestinal motility [6-8]. The effects on energy balance are manifested in body weight gain and adiposity, probably because ghrelin stimulates food intake and suppresses fat metabolism and energy expenditure [2-5]. Ghrelin is synthetized in the so-called A-like cells in the gastric mucosa [9,10] and secreted in response to nutritional deficiency [2,10-13]. Nutrient-dependent signals suppress the release of ghrelin [13-17]. Although it is likely that neurocrine, paracrine and endocrine signals participate in the regulation of ghrelin secretion, available evidence suggests that such signals play a relatively minor role [18].

Ghrelin is a 28-amino acid peptide, generated by the processing of proghrelin [1,19]. During processing, serine in position 3 in desacyl ghrelin is octanoylated to form bioactive acylated ghrelin [1,19,20]. Until recently, desacyl ghrelin was claimed to be inactive. Interestingly, desacyl ghrelin predominates over acyl ghrelin in the rat stomach [20] and accounts for almost 90 % of circulating ghrelin in both rat [20] and man [21]. This observation is surprising and difficult to reconcile with the view that only acylated ghrelin is biologically active. Indeed, desacyl ghrelin was recently claimed to act in conjunction with acyl ghrelin, having inverse effects, decreasing food intake and delaying gastric emptying [22]. Muccioli *et al* [23], on the other hand, reported that both acyl ghrelin and desacyl ghrelin antagonized lipolysis in rat adipocytes. Recently, it was reported that cleavage of proghrelin may generate another bioactive fragment consisting of 23 amino acid residues, named obestatin [24]. This peptide was claimed to suppress food intake and to inhibit gastrointestinal motility, contrary to the effects of ghrelin [24,25, for a different view see 26]. Interestingly, the plasma obestatin concentration was reported to be similar to that of acyl ghrelin [24].

The effects of ghrelin (i.e. acyl ghrelin) on insulin and glucagon secretion have been studied by several investigators [27-33]. However, results in the past have been

contradictory, probably because of differences with respect to the doses given and the animal species tested [9,27-33]. In pancreatic islets of the mouse, acyl ghrelin was found to inhibit insulin secretion at low doses and to stimulate at high doses; glucagon secretion on the other hand was stimulated at both low and high doses [32]. In pancreatic islets of the rat, acyl ghrelin was found to inhibit the secretion of insulin and to stimulate that of glucagon [33]. An inhibitory effect of acyl ghrelin on the release of pancreatic somatostatin has been noted [29], but the response of pancreatic polypeptide (PP) to acyl ghrelin has not yet been investigated.

In view of the possibility that acyl ghrelin is released together with desacyl ghrelin and obestatin, it seemed reasonable to explore the combined effects of the proghrelin-derived peptides on the secretion of hormones from the pancreatic islets. We have therefore examined the effects of acyl ghrelin (active ghrelin), desacyl ghrelin (previously thought to be inactive) and obestatin, alone and together, on the secretion of insulin, glucagon, PP and somatostatin from isolated pancreatic islets of mice and rats. We studied islets from the splenic and duodenal lobes of the pancreas separately. Islets from the splenic part are rich in glucagon cells, whereas those from the duodenal part are rich in PP cells [34].

2. Materials and methods

2.1. Chemicals

Collagenase (CLS-4) from Sigma (Freehold, NJ, USA) was used to prepare the pancreatic islets. Bovine serum albumin (BSA) was from ICN Biomedical (High Wycombe, UK). Rat ghrelin (acyl ghrelin 1-28) and desacyl ghrelin were generously supplied by Professor Chizuka Yanaihara at the Yanaihara Institute, Shizuoka, Japan. Rat obestatin (1-23) was purchased from GL Biochem (Shanghai, China). All other chemicals were from British Drug Houses (Poole, UK) or Merck (Darmstadt, Germany).

2.2. Animals

Female mice of the NMRI strain (B&K Universal, Sollentuna, Sweden), weighing 25-30 g, and female rats of the Sprague Dawley strain, weighing 200-220 g, were used in the studies. They were fed a standard pellet diet (B&K Universal) and tap water *ad libitum.*

The investigation, which involved the collection of fresh tissue material from animals killed without anaesthesia, was approved by the local animal welfare committee. The animals were killed by cervical dislocation (mice) or by decapitation (rats). A collagenase solution was immediately injected into the bile-pancreatic duct to distend the pancreas, followed by excision of the pancreas. The islets were isolated by a standard digestion procedure [35 from the splenic and duodenal lobes separately (unless otherwise stated). The islets were collected at room temperature using a stereomicroscope. Each splenic lobe from the mouse yielded 200-250 islets and each duodenal lobe 50-70 islets. Each splenic lobe from the rat yielded 300-350 islets and each duodenal lobe 70-100 islets. Each batch of islets consisted of freshly isolated islets that were pooled from 3-4 animals. Aliquots of such batches were used for each experiment (e.g. dose/concentration response experiments). Some

of the islets were collected for measurement of the islet content of insulin, glucagon, PP and somatostatin (see below). Others were preincubated (12 islets in a volume of 1 ml) for 30 min at 37ºC in Krebs-Ringer bicarbonate buffer, pH 7.4, supplemented with 10 mM Hepes, 0.1 % bovine serum albumin and 1 mM glucose. Each incubation vial was gassed with 95 % 0_2 and 5 % CO_2 to obtain constant pH and satisfactory oxygenation. After preincubation, the buffer was changed to a medium that contained different concentrations of the peptides to be tested (acyl ghrelin, desacyl ghrelin, obestatin) at 12 mmol glucose per liter (mouse islets) or 8.3 mmol glucose per liter (rat islets). It is well known that the glucose dose-response curve for rat islets with regard to insulin release is shifted to the left *versus* mouse islets. Hence, the glucose doses used in the present study were different for rat and mouse islets; the two doses were chosen because they gave the same insulin response. The samples were incubated in a metabolic shaker (30 cycles per min) for 60 minutes at 37ºC. Immediately after incubation, the islets were sedimented and aliquots of the medium (350 µl) were removed for the radioimmunoassay (RIA) of insulin, glucagon, PP, and somatostatin.

For measurement of the concentration of the various islet hormones, the collected islets (portions of 50 islets in 200 µl acetate-EDTA buffer: 1.1 mmol/l EDTA and 5 mmol/l sodium acetate, pH 5.0) were sonicated on ice. Aliquots (10 µl) of the homogenates were subjected to protein determination [36]. Other aliquots (20 µl) of the homogenates were extracted with 2 ml acid ethanol (96 vol. ethanol, 2.4 vol. conc. H_2SO_4, 18 vol. water) overnight [37] and the clear supernatants were analysed for insulin, glucagon, PP and somatostatin by RIA (see below).

2.3. Design of study

1) Mouse and rat islets were incubated with increasing concentrations of acyl ghrelin, desacyl ghrelin or obestatin. Islets from the splenic part of the pancreas were compared

with islets from the duodenal part. A stimulating level of glucose in the medium is thought to be required for the demonstration of an effect of acyl ghrelin on insulin secretion [31].

2) Mouse islets were incubated with acyl ghrelin (10^{-10} M) plus desacyl ghrelin (10^{-9} M). This combination was chosen because acyl ghrelin inhibits insulin secretion from mouse islets at that dose [32], because the circulating concentration of total ghrelin (i.e. acyl ghrelin + desacyl ghrelin) in the mouse and rat is in the range 10^{-10}-10^{-8} M [1,2,4,10,12,13,14,18,29,30], and because of reports that almost 90 % of circulating ghrelin is desacyl ghrelin in both rats [20] and patients [21]. Rat islets were incubated with acyl ghrelin (10^{-8} M) + desacyl ghrelin (10^{-7} M). This concentration of acyl ghrelin was chosen because it inhibits insulin secretion from rat islets [33]. Islets were also incubated with obestatin (mouse islets: 10^{-10} M, rat islets 10^{-8} M) alone and together with acyl ghrelin or desacyl ghrelin (concentrations as above). Obestatin is reported to circulate in a concentration corresponding to 10 % of the total ghrelin concentration (acyl ghrelin + desacyl ghrelin) [24], and equimolar amounts of obestatin and acyl ghrelin were combined with 10 times higher concentration of desacyl ghrelin (because desacyl ghrelin predominates greatly over acyl ghrelin in the circulation [20,21]). In these experiments, the islets were from the whole pancreas.

2.4. Hormone measurements

The concentrations of insulin, glucagon, somatostatin and PP in the incubation medium were measured by radioimmunoassay (RIA). RIA kits for insulin were obtained from Diagnostika (Falkenberg, Sweden) and for glucagon and somatostatin from Eurodiagnostica (Malmö, Sweden). The antiserum used in the glucagon assay binds pancreatic glucagon but neither of the intestinal glucagon-like peptides-1 and -2. PP was determined by a RIA kit from Linco Research (St. Charles, MO, USA) that measures mouse and rat PP with the same accuracy.

2.5. Statistical analysis

Results were expressed as means ±SEM, n being the number of independent experiments. Each observation is the mean of two measurements on aliquots containing 12 islets from one and the same batch of islets. The level of significance for the difference between sets of data was assessed using Student's unpaired t-test or analysis of variance followed by Tukey-Kramers test whenever appropriate. $P<0.05$ was considered statistically significant. The concentration of the proghrelin products that caused 50 % of maximum release (glucagon) is referred to as the EC_{50} value. The concentration of the peptides that suppressed hormone release (insulin, PP, somatostatin) by 50 % of maximum inhibition is referred to as IC_{50}. The EC_{50} and IC_{50} values were calculated from the concentration/dose-response curves by using a computer software aided curve fitter, the GraphPad PRISM programme (version 3.00, GraphPad Software, San Diego, CA, USA).

3. **Results**

3.1. Glucose-stimulated release of hormones from islets isolated from the splenic part versus the duodenal part of the pancreas

Islets in the duodenal lobe of the pancreas are known to differ from islets in the splenic lobe by having fewer glucagon cells and many more PP cells [34]. In fact, there are virtually no PP cells in the splenic lobe. This characteristic difference between islets from the splenic and the duodenal parts of the pancreas was corroborated in the present study by measurement of hormone concentrations in the islets (Fig 1A and B). Hormone secretion in response to glucose in the medium, in the absence of added proghrelin-derived peptides (Fig 1C and D), reflected the relative preponderance of glucagon in splenic islets and of PP in duodenal islets. The release of glucagon from splenic islets was 2-3-fold greater than from duodenal islets, while the release of PP was more than ten-fold higher from duodenal islets than from splenic islets. On the whole, the release of insulin and somatostatin did not differ much between islets from the two parts of the pancreas. The hormone contents and the hormone release patterns were quite similar in mouse and rat islets (Fig 1C and D).

3.2. Release of islet hormones in response to acyl ghrelin, desacyl ghrelin and obestatin alone and in various combinations

3.2.1. Effects of acyl ghrelin

Islets were challenged with increasing amounts of acyl ghrelin, and the secretion of insulin, glucagon, PP and somatostatin was monitored. Mouse islets responded to 10^{-10} M acyl ghrelin with a modest inhibition of insulin secretion (see also [32]), while insulin secretion was stimulated at 10^{-6} M concentration (Fig. 2). The resulting concentration-response curve seemed to consist of two curves superimposed on each other, one showing inhibition of insulin release and the other showing stimulation. Rat islets responded to acyl

ghrelin with a dose-dependent, modest inhibition of insulin secretion; quite high concentrations were required to cause inhibition ($\geq 10^{-8}$ M) (Fig 3); there was no stimulation of insulin secretion at any of the concentrations tested (see also [33]). In both mouse and rat islets, acyl ghrelin stimulated glucagon secretion concentration-dependently, while it inhibited the secretion of PP and somatostatin (Figs. 2 and 3). The response pattern was similar in islets from both the splenic and the duodenal lobes, except that islets from the duodenal lobe released less glucagon and much more PP than islets from the splenic lobe.

EC_{50} values (for glucagon release) and IC_{50} values (for inhibition of insulin, PP and somatostatin release) are shown in Table 1. The IC_{50} value for acyl ghrelin-induced inhibition of insulin release ($7.5x10^{-8}$ M in rat islets) was about two orders of magnitude higher than the EC_{50} value for glucagon release and the IC_{50} values for inhibition of PP and somatostatin release ($3x10^{-12}$ - $15x10^{-12}$ M).

3.2.2. Effects of desacyl ghrelin

Desacyl ghrelin in the concentration range tested was without effect on the secretion of the four pancreatic hormones from islets of either mice or rats (Figs 2 and 3).

3.2.3. Effects of obestatin

The secretion of insulin was inhibited by obestatin in a concentration-dependent manner in both mouse and rat islets, while the secretion of glucagon was stimulated. Obestatin, moreover, inhibited the secretion of both somatostatin and PP, much like acyl ghrelin (Figs 2 and 3).

The EC_{50} values for glucagon release ($9.3x10^{-12}$ M and $5.5x10^{-12}$ M) were found to be in the same range as the IC_{50} values for inhibition of insulin, PP and somatostatin release ($4x10^{-12}$ - $20x10^{-12}$ M) (Table 1).

3.2.4. Effects of proghrelin-derived peptides in conjunction

The combination of acyl ghrelin and a 10 times higher dose of desacyl-ghrelin abolished the effects of acyl ghrelin on the secretion of all four pancreatic hormones in islets from both mice and rats (Figs 4 and 5). The combination of obestatin and 10 times higher concentration of desacyl ghrelin resulted in islet hormone responses that were very similar to those observed after challenge with obestatin alone. This was the case with islets from both mice and rats (Figs 4 and 5). The experiments summarized in Figs 4 and 5 were conducted on islets from the whole pancreas; in this preparation approximately 75-80 % of the islets were from the splenic lobe (see methods 2.2).

4. Discussion

Available evidence suggests that acyl ghrelin derives from the A-like cells in the stomach [9,10], and that proghrelin, a polypeptide of 94 amino acid residues [1,19], is packaged in the small, round, electron-dense secretory granules that are characteristic of these cells [38]. Proghrelin undergoes intragranular cleavage to generate a 28-amino acid peptide, desacyl ghrelin, which is octanoylated in the serine 3-position [1,19]. The resulting acyl ghrelin is an orexigenic and adipogenic peptide hormone, thought to initiate food intake and to control adipogenesis and energy expenditure [1-5]. According to Zhang *et al* [24] the remaining C-terminal end of the proghrelin molecule is processed to generate a carboxyamidated 23-amino acid peptide named obestatin. This peptide is said to act as a physiological antagonist to acyl ghrelin [39] in that it is claimed to decrease food intake and body weight gain in rodents [24,25]. Although acyl ghrelin is generally thought to be the active form of ghrelin, desacyl ghrelin (for long thought to be inactive) seems to represent the quantitatively predominant form of ghrelin both in ghrelin cells [20] and in circulation [20,21]. In fact, however, the three products of proghrelin all seem to be bioactive, not only acyl ghrelin but also desacyl ghrelin [22,23] and obestatin [24-26]. Despite preliminary evidence to the contrary [20], it is to be expected that all secretory products will be released concomitantly from the A-like cells, i.e. ghrelin peptides together with obestatin (conceivably in stoichiometric amounts). Consequently, investigating the combined effects of these peptides may be of greater interest than investigating the effect of each peptide individually.

While the effects of obestatin on the release of insulin and glucagon have not been studied in any detail so far [see e.g. ref 38], the effects of acyl ghrelin have been studied quite extensively [27-33]. Acyl ghrelin inhibits insulin release and stimulates glucagon release from isolated pancreatic islets from both mice and rats [31-33]. In fact, however, acyl ghrelin was found to have a complex effect on insulin release in that secretion in mouse islets was

inhibited at low to moderate doses (10^{-12}-10^{-10} M) and stimulated at high doses (>10^{-8} M) [32]. On the whole, our results are in line with those of Kvist Reimer *et al* [31], who showed a U-shaped dose-response relationship between the ghrelin dose and the inhibition of insulin secretion. Rat islets, on the other hand, were found to respond to high concentrations of acyl ghrelin (≥10^{-8} M) with a modest inhibition of insulin release. In contrast, Date *et al* [9] reported that rat islets responded to acyl ghrelin (10^{-12} M) with a stimulatory effect on glucose-induced insulin release. At present, we have no explanation for this discrepancy.

We decided to study first the effects of acyl ghrelin, desacyl ghrelin and obestastin individually on the release of insulin and glucagon as well as on the release of PP and somatostatin and then to study the effects of the proghrelin products given concomitantly. We could show that while acyl ghrelin at 10^{-10} M concentration inhibited insulin secretion from islets of both the splenic and the duodenal lobes of the mouse, high concentrations of acyl ghrelin (>10^{-8} M) stimulated insulin release [see 32], and we could confirm also the inhibitory effect of acyl ghrelin on insulin secretion from rat islets [33]. We could show also that acyl ghrelin stimulated glucagon secretion from both splenic and duodenal islets of both mice and rats [32,33]. Moreover, we observed that acyl ghrelin inhibited the secretion of PP as well as that of somatostatin. Desacyl ghrelin, on the other hand, did not affect the secretion of any of the pancreatic hormones. The EC_{50} and IC_{50} values for acyl ghrelin with respect to glucagon release and inhibition of PP and somatostatin release were quite similar, and it seems conceivable therefore that a similar type of receptor is responsible for the effects in these three islet cell types. The IC_{50} value for inhibition of insulin release, however, differed by about two orders of magnitude, suggesting the involvement of a different acyl ghrelin receptor with low affinity. The effects of obestatin on the various islet cells were quite similar to the effects of acyl ghrelin, except that obestatin, but not acyl ghrelin, inhibited insulin release at physiologically relevant concentrations. The values for EC_{50} (glucagon release) and

IC_{50} (insulin, PP, somatostatin) for obestatin were all in the same range, suggesting that the receptor(s) responsible for the obestatin-induced effects had similar properties in the different islet cell types.

The suppression of insulin release by acyl ghrelin is in line with several earlier reports [27,29,30,32,33]. Hence, although the effect was weak at physiologically relevant concentrations, acyl ghrelin may operate as a negative rather than a positive modulator of the insulin response to glucose. Indeed, Egido *et al* [29] suggested that the inhibitory effect of acyl ghrelin on insulin release might constitute a tonic regulation of the insulin cell, restraining its activity in a state of food deprivation. Whether the effect reflects a direct action of acyl ghrelin on the insulin cells or whether simultaneous changes in the secretion of glucagon, PP or somatostatin contribute to the altered insulin secretion is not known. The addition of ten times higher concentration of desacyl ghrelin than of acyl ghrelin abolished the effects of acyl ghrelin on the islets, suggesting that desacyl ghrelin interferes with acyl ghrelin binding to islet cell receptors. The effects of obestatin, which were manifested at physiologically relevant concentrations, were not inhibited by desacyl ghrelin. The combination of obestatin with equimolar amounts of acyl ghrelin and with a ten times higher dose of desacyl ghrelin resulted in hormonal responses that were indistinguishable from those to obestatin alone, suggesting that ghrelin peptides do not interfere with the obestatin receptor and *vice versa*. Although the proghrelin-derived peptides, alone and/or together, affected the secretion of all four islet hormones, it is not possible to conclude from the present findings that these effects reflect a physiological role. Nonetheless, the effective concentration range of the different proghrelin-derived peptides is such that they may well contribute to the regulation of islet hormone secretion. Both acyl ghrelin and obestatin may play a role in stimulating the activity of the glucagon cells and restraining the activities of the insulin, PP and somatostatin cells in the pancreas in situations characterized by nutritional deficiency.

It may be speculated that all anticipated effects of endogenous acyl ghrelin (on for instance appetite control, adipogenesis, energy expenditure, and gut peristalsis) may be compromised by concomitantly mobilized desacyl ghrelin and obestatin. Conceivably, the actions of endogenous, circulating acyl ghrelin may be blunted by desacyl ghrelin and/or overshadowed by the effects of obestatin.

Acknowledgements

The study was supported by grants from the Swedish Research Council (grant K2006-04x), the Diabetes program (the Medical Faculty, University of Lund), the Albert Påhlsson Foundation, the Golje Foundation and the Novo Nordisk Foundation.

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Legends to Figures

Fig. 1. Concentrations of insulin, glucagon, pancreatic polypeptide (PP) and somatostatin in freshly isolated islets from the splenic and duodenal parts of the mouse and rat pancreas, expressed as amoles per µg islet proteins (A and B). Secretion of insulin, glucagon, PP and somatostatin from such islets in response to 12 mmol glucose per liter (mouse islets) or 8.3 mmol glucose per liter (rat islets), expressed as amol hormone released per islet and per hour (C and D). Mean values ±SEM (vertical bars), n=10 (mouse) and 12 (rat) batches of islets. Student´s unpaired t-test: Statistical significance of differences between islets from splenic and duodenal lobes is indicated by * for $P<0.001$.

Fig. 2. Secretion of insulin, glucagon, pancreatic polypeptide (PP) and somatostatin from mouse islets (collected from the splenic or the duodenal parts of the pancreas) in response to increasing concentrations of acyl ghrelin, desacyl ghrelin and obestatin in the presence of 12 mmol glucose per liter. Each point is the mean of 10 observations (from 10 independent dose-response experiments). Mean values ±SEM (vertical bars)

Fig. 3. Secretion of insulin, glucagon, pancreatic polypeptide (PP) and somatostatin from rat islets (collected from the splenic or the duodenal parts of the pancreas) in response to increasing concentrations of acyl ghrelin, desacyl ghrelin and obestatin in the presence of 8.3 mmol glucose per liter. Each point is the mean of 8 observations (from 8 independent dose-response experiments) Meran values ±SEM (vertical bars).

Fig. 4. Secretion of insulin, glucagon, pancreatic polypeptide (PP) and somatostatin from mouse islets in response to acyl ghrelin (10^{-10} M), obestatin (10^{-10} M) and desacyl ghrelin (10^{-9} M), alone and in various combinations as indicated. Glucose concentration in the medium was 12 mmol per liter. Islets were collected from whole pancreas. The secretion rate is expressed as amoles of the respective hormone released per islet per h. Mean values ±SEM

(vertical bars), n=10 batches of fresh islets from 10 different animals killed on different occasions. Analysis of variance:* for $P<0.001$

Fig. 5. Secretion of insulin, glucagon, pancreatic polypeptide (PP) and somatostatin from rat islets in response to acyl ghrelin (10^{-8} M), obestatin (10^{-8} M) and desacyl ghrelin (10^{-7} M), alone and in various combinations, as indicated. Glucose concentration in the medium was 8.3 mmol per liter. Islets were collected from whole pancreas. The secretion rate is expressed as amoles of the respective hormone released per islet per h. Mean values ±SEM (vertical bars), n=12 batches of fresh islets from 12 different animals killed on different occasions. Analysis of variance:* for $P<0.001$.

Table 1: Effects of proghrelin-derived peptides on pancreatic hormone secretion: EC_{50} and IC_{50} values.

Mouse islets

Proghrelin product	Insulin release IC_{50} (M)	Glucagon release EC_{50} (M)	PP release IC_{50} (M)	Somatostatin release IC_{50} (M)
Acyl ghrelin	a	10.7×10^{-12} M (±3.2)	9.2×10^{-12} M (±3.1)	7.4×10^{-12} M (±2.3)
Desacyl ghrelin	-	-	-	-
Obestatin	20×10^{-12} M (±5.1)	9.3×10^{-12} M (±2.4)	9.5×10^{-12} M (±3.7)	14.4×10^{-12} M (±3.9)

Rat islets

Proghrelin product	Insulin release IC_{50} (M)	Glucagon release EC_{50} (M)	PP release IC_{50} (M)	Somatostatin release IC_{50} (M)
Acyl ghrelin	7.5×10^{-8} M (±2.7)	15×10^{-12} M (±3.0)	5×10^{-12} M (±1.6)	3×10^{-12} M (±2.2)
Desacyl ghrelin	-	-	-	-
Obestatin	10.7×10^{-12} M (±2.5)	5.5×10^{-12} M (±2.3)	4.1×10^{-12} M (±1.2)	6.3×10^{-12} M (±2.1)

Means ±SEM (in brackets), n=10 (mouse islets) and 8 (rat islets)

- \- indicates no effect.
- a The insulin dose-response curve was found to be complex/incomplete (probably composed of two curves superimposed upon each other) (Fig 2) and hence, the IC_{50} value could not be calculated.

Notes to the Table. EC_{50} and IC_{50} values were calculated from the data that were used to construct the concentration response curves shown in Figs 2 and 3. These curves summarized 10 (mouse islets) and 8 (rat islets) separate concentration response curves, each used to calculate EC_{50} or IC_{50} values. These individual values were then used to calculate the mean values shown in the Table. Acyl ghrelin and obestatin stimulated glucagon secretion (EC_{50} values were calculated, response minus baseline) and inhibited the secretion of insulin, PP and somatostatin (IC_{50} values were calculated). Desacyl ghrelin was without effect. EC_{50} values for glucagon release were calculated using data from islets of the splenic lobe. IC_{50} values were calculated using data from splenic islets (insulin and somatostatin release) or from duodenal islets (PP release).

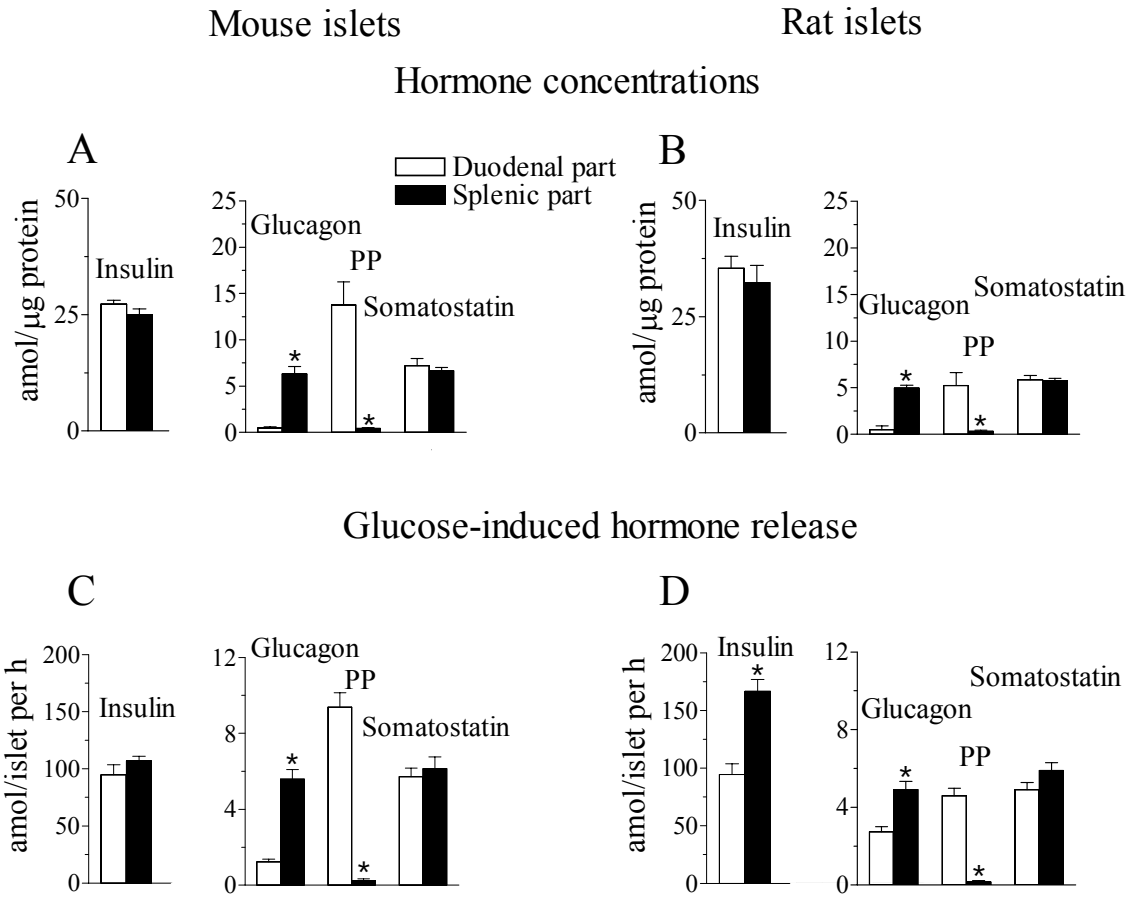
Mouse islets
Rat islets
Hormone concentrations
A
B
Duodenal part
Splenic part
amol/µg protein
Insulin
Glucagon
PP
Somatostatin
Glucose-induced hormone release
C
D
amol/islet per h

Fig 1

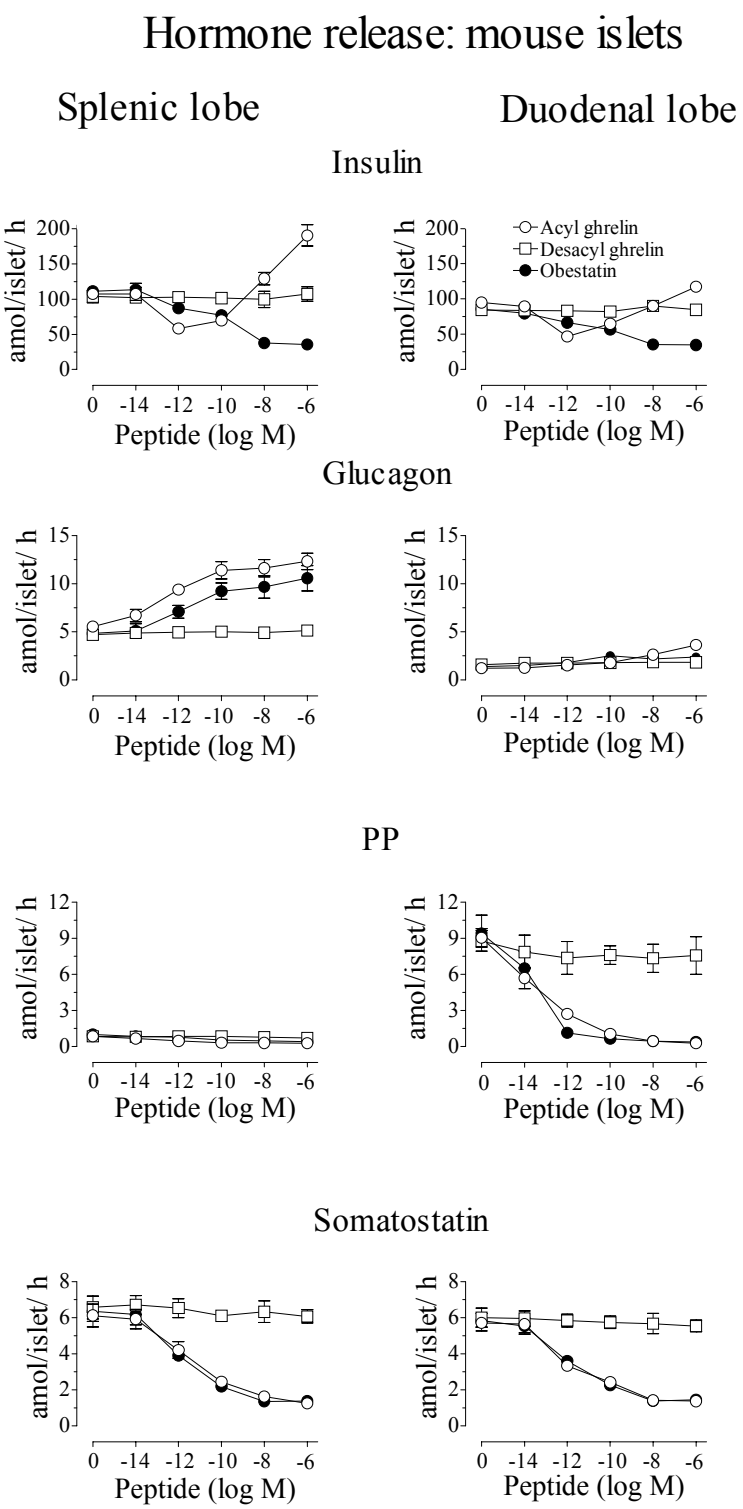
Hormone release: mouse islets
Splenic lobe
Duodenal lobe
Insulin
Glucagon
PP
Somatostatin
amol/islet/ h
Peptide (log M)
0 -14 -12 -10 -8 -6
Acyl ghrelin
Desacyl ghrelin
Obestatin

Fig 2

Hormone release: rat islets

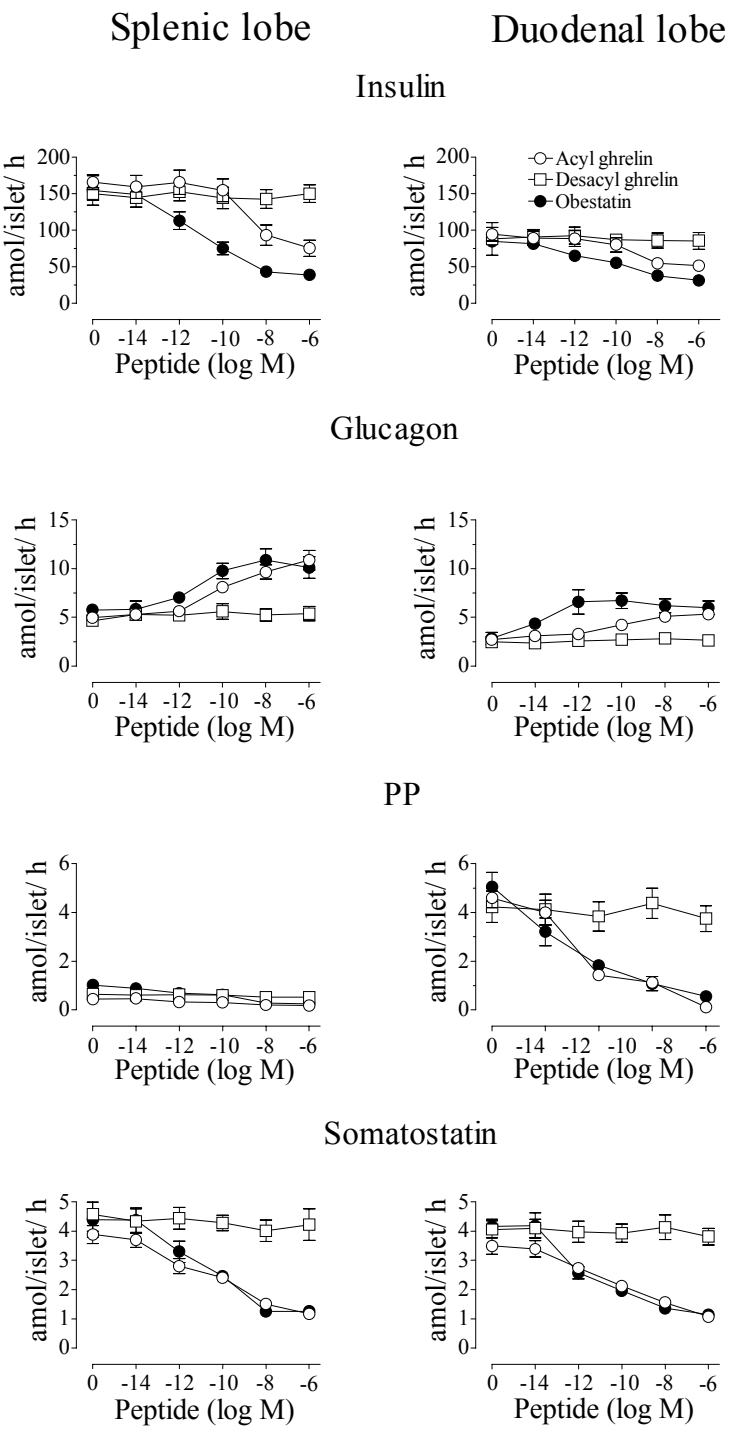

Fig 3

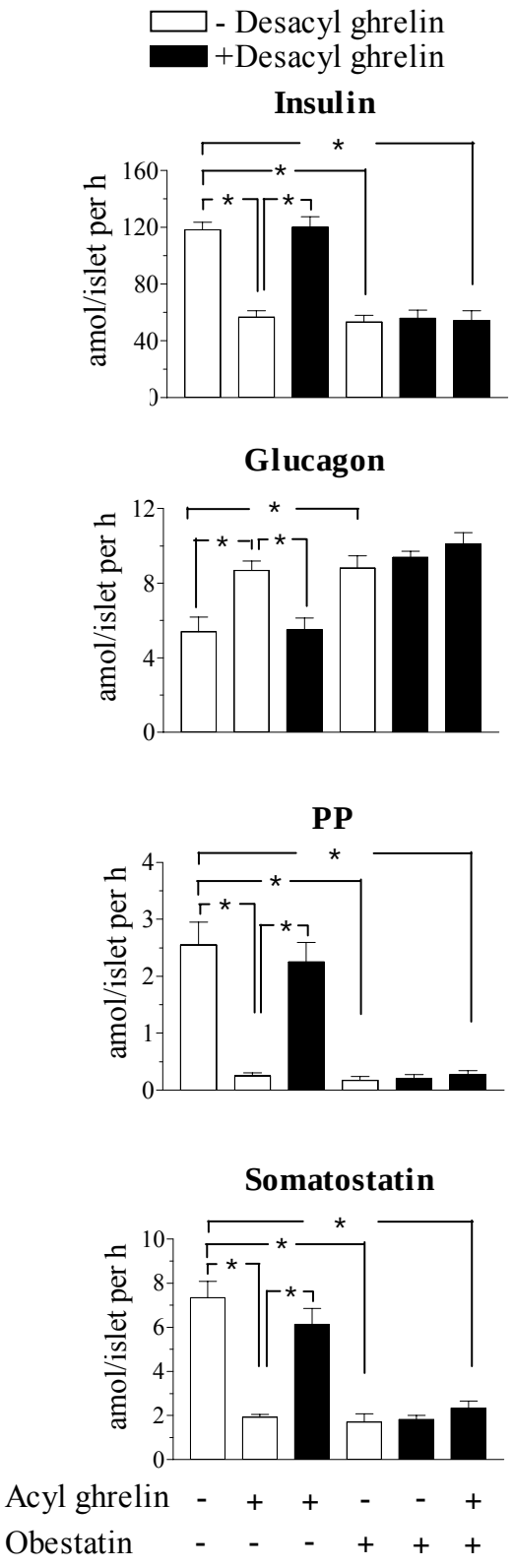
Hormone release: mouse islets
- Desacyl ghrelin
+Desacyl ghrelin
Insulin
amol/islet per h
160
120
80
40
0
Glucagon
amol/islet per h
12
8
4
0
PP
amol/islet per h
4
3
2
1
0
Somatostatin
amol/islet per h
10
8
6
4
2
0
Acyl ghrelin - + + - - +
Obestatin - - - + + +

Fig 4

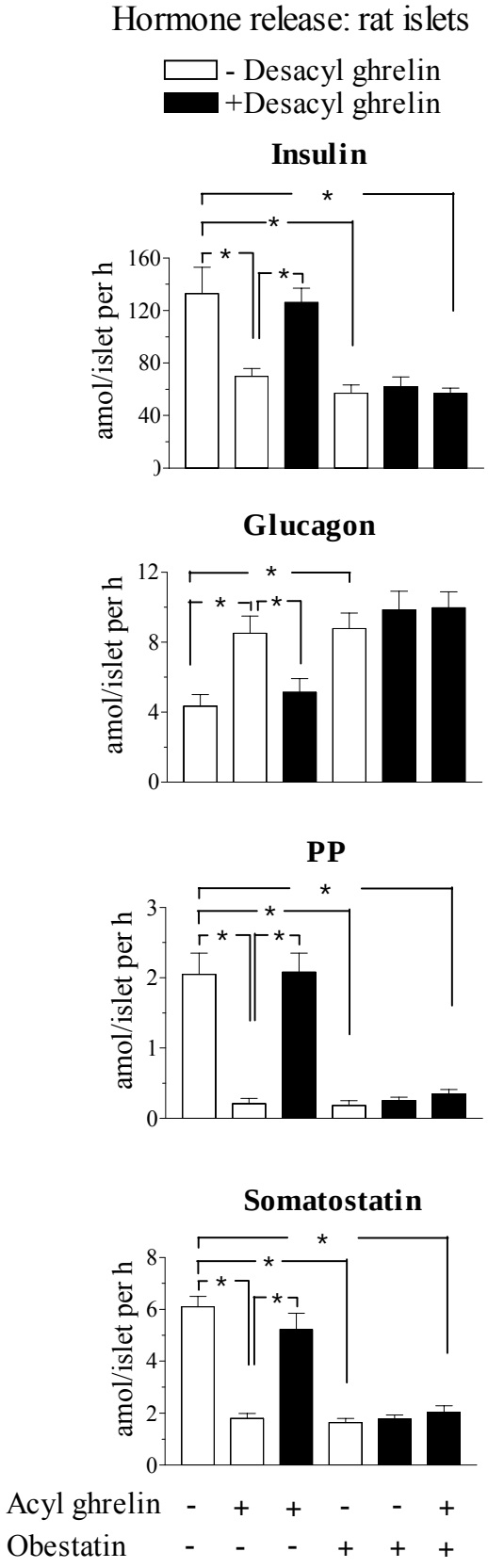
Hormone release: rat islets
- Desacyl ghrelin
+Desacyl ghrelin
Insulin
amol/islet per h
160
120
80
40
Glucagon
amol/islet per h
12
8
4
0
PP
amol/islet per h
3
2
1
0
Somatostatin
amol/islet per h
8
6
4
2
0
Acyl ghrelin - + + - - +
Obestatin - - - + + +

Fig 5