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Proghrelin peptides: Desacyl ghrelin is a powerful inhibitor of acylated ghrelin, likely to impair physiological effects of acyl ghrelin but not of obestatin.
A study of pancreatic polypeptide secretion from mouse islets

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Short title: Effect of proghrelin peptides on PP release

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Abstract

Background. Proghrelin, produced by the ghrelin (A-like) cells of the gastric mucosa, gives rise to cleavage products, including desacyl ghrelin, acyl ghrelin and obestatin. The products are thought to be secreted concomitantly. In an earlier study we found acyl ghrelin and obestatin, but not desacyl ghrelin, to suppress the release of hormones from isolated islets of mouse and rat pancreas.
Results. Using isolated mouse pancreatic islets to study the suppression of the spontaneous secretion of pancreatic polypeptide (PP) by acyl ghrelin and obestatin, we determined the EC_{50} values for the two peptides. For acyl ghrelin it was 2×10^{-13} M (ranging from 1.7 to 2.8×10^{-13} M), for obestatin it was 10^{-13} M (ranging from 0.3 to 1.1×10^{-13} M). The Hill coefficient (i.e. the midpoint slope) for the acyl ghrelin dose-response curve was 0.30 (ranging from 0.21 to 0.35); the corresponding value for obestatin was 0.35 (ranging from 0.21 to 0.35). The PP-releasing effect of acyl ghrelin, but not that of obestatin, was counteracted by desacyl ghrelin. The acyl ghrelin dose-response curve was shifted to the right in a parallel manner by increasing concentrations of desacyl ghrelin. A Schild plot was constructed with a slope of 0.78, giving an apparent pA_2 value of 14.
Conclusions. The results favour the view that acyl ghrelin and obestatin suppress spontaneous PP secretion at physiologically relevant concentrations and that they act on separate receptors. However, we conclude also that desacyl ghrelin acts as a competitive, surmountable (and quite potent) inhibitor of acyl ghrelin. In view of the allegedly high circulating concentrations of desacyl ghrelin it is to be expected that the effect of acyl ghrelin – but not that of obestatin - will be impaired, in fact probably severely blunted by desacyl ghrelin, thereby compromising the functional significance of circulating acyl ghrelin. In addition, we suggest that isolated pancreatic islets are well suited for studies of receptors to acyl ghrelin and obestatin, and that suppression of PP secretion represents a convenient way to measure the effect of both these peptides.

Keywords: Ghrelin; Acyl ghrelin; Desacyl ghrelin; Obestatin; Pancreatic polypeptide; PP, PP cells

Abbreviations: PP, pancreatic polypeptide; RIA, radioimmunoassay

1. **Introduction**

Desacyl ghrelin (28 a.a. residues) and obestatin (23 a.a.) are cleavage products of proghrelin (1,2), which is synthetized in endocrine A-like cells in the gastric mucosa (3,4). During processing, a fraction of desacyl ghrelin is acylated in position 3 (serine) to form variants of acylated ghrelin (1,2,5-7), which are considered to be biologically active. It is noteworthy that desacyl ghrelin is more abundant than acyl ghrelin in both stomach (5) and circulation (5,8-10). Conceivably, desacyl ghrelin is being produced in the ghrelin cells as well as in the circulation by deacylation of acylated ghrelin. Being cleavage products of proghrelin, obestatin and the ghrelin peptides are likely to be released together from the A-like cells, conceivably affecting their targets in concert. Interestingly, secretion of ghrelin seems to be regulated by circulating signals rather than by gastric signals (11).

Acyl ghrelin is thought to be a "hunger hormone" because circulating ghrelin concentrations increase as a result of food deprivation and decrease following food intake (1,4,8-15). Numerous reports have suggested that ghrelin affects energy balance; causing body weight increase and adiposity (see *e.g.*16, 17). More specifically, acyl ghrelin has been found to initiate food intake and to suppress fat metabolism and energy expenditure (18-20). Desacyl ghrelin has been claimed to be inactive (1,2). Obestatin, on the other hand, has been found to suppress food intake (21,22), contrary to the effect of acyl ghrelin (23), but other studies have failed to confirm this (24,25). Consequently, the effects of obestatin on food intake, gastric motility and energy balance are still controversial, and the major targets for both ghrelin and obestatin remain to be identified. Indeed, the physiological relevance of both acyl ghrelin and obestatin is far from clear.

The effects of the three proghrelin-derived peptides on the release of metabolically active hormones have been studied quite extensively in the past. However, while the effects of acyl ghrelin on insulin, glucagon and somatostatin secretion have attracted some interest (26-31), few laboratories have examined the effects of the proghrelin-derived peptides on the fourth islet hormone pancreatic polypeptide (PP). In healthy volunteers, injection of ghrelin was found to raise circulating levels of PP (32).

From studies of isolated islets of rat and mouse pancreas it seems that acyl ghrelin stimulates glucagon secretion and suppresses the secretion of insulin, somatostatin and PP (31). In mouse islets, acyl ghrelin was found to suppress insulin release at low doses and to stimulate at high, suggesting the existence of at least two distinct acyl ghrelin receptors (31). Surprisingly, the effects of obestatin on islet hormone secretion seemed to be quite similar to those of acyl ghrelin, causing suppression of insulin, somatostatin and PP release, while stimulating glucagon release. Desacyl ghrelin *per se* had no effect on islet hormone secretion (31).

The PP-suppressive effects of acyl ghrelin and obestatin were found to be quite powerful, and we decided therefore to make use of isolated mouse islets to examine 1) whether the known proghrelin peptides act on separate receptors to suppress spontaneous PP release and 2) whether they can be expected to play a physiologically relevant role in controlling the release of PP (and conceivably other islet hormones). 3) In addition, we wished to examine if and how desacyl ghrelin interferes with responses to acyl ghrelin and obestatin.

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, i.e. n-octanoyl 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. *Preparation of islets from mouse pancreas*

Female mice of the NMRI strain (B&K Universal, Sollentuna, Sweden), weighing 25-30 g, were used. They were fed a standard pellet diet (B&K Universal) and tap water *ad libitum* until they were killed by cervical dislocation. A collagenase solution was immediately injected into the bile-pancreatic duct to distend the pancreas, followed by excision of the duodenal, PP-rich part of the pancreas (31). The islets were isolated by a standard digestion procedure (33) and collected at room temperature using a stereomicroscope. Each pancreas (duodenal part) yielded 80-100 islets.

Each batch of islets consisted of freshly isolated islets from 8-10 mice. Aliquots of such batches were used for each concentration-response experiment. Each aliquot was preincubated (12 islets in a volume of 1 ml) for 30 min in an incubation vial at 37^{o} C in Krebs-Ringer bicarbonate buffer, pH 7.4, supplemented with 10 mM Hepes, 0.1 % BSA and 1 mmol glucose. During preincubation, each vial was gassed with 95 % O_2 and 5 % CO_2 to obtain constant pH and satisfactory oxygenation. The islets sedimented to the bottom of the tube during this process. After preincubation, the buffer was changed to a medium that contained different concentrations of the peptides to be tested (acyl ghrelin, desacyl ghrelin, obestatin – alone or in various combinations) at 12 mmol glucose per liter. The incubation volume was 1 ml. The samples were incubated in a metabolic shaker (30 cycles per min) for 60 min at 37^{o} C. Immediatelythereafter, the islets were sedimented and aliquots of the medium (350 µl) were removed for radioimmunoassay (RIA) of PP.

2.3. *Design of study*

Mouse islets were incubated with increasing concentrations of acyl ghrelin or obestatin, alone or together with desacyl ghrelin. Each observation is the mean of two measurements on aliquots containing 12 islets from one and the same batch of islets. All dose-response curves were obtained under identical experimental conditions (see 2.2.).

2.4. *PP measurement*

The PP concentration in the incubation medium was measured by RIA, using a kit from Linco Research (St. Louis, MO, USA) (31).

2.5. Data collection and analysis

Acyl ghrelin, obestatin and desacyl ghrelin are referred to as agonists (or potential agonists) in the first part of the study involving concentration-response curves. The fact that acyl ghrelin and obestatin suppress PP release do not disqualify them from being referred to as agonists. Desacyl ghrelin is referred to as antagonist (because during the course of the study it was found to inhibit the response to acyl ghrelin) in the second part of the study in which we examined the effects of combinations of acyl ghrelin and desacyl ghrelin on one hand and of obestatin and desacyl ghrelin on the other.

2.5.1. Agonist-induced suppression of PP release: Concentration-response curves were constructed illustrating the suppression of spontaneous PP release induced by the agonists acyl ghrelin and obestatin. Also the effect of desacyl ghrelin was tested in the same fashion. The data from each concentration-response curve was fitted to a sigmoidal curve to allow us to calculatethe maximum suppressive response, the Hill coefficient and the EC_{50} value. Curves were constructed based on actual numerical data as well as normalized data. In the latter case data from each dose-response experiment were normalised, 100% representing PP release at zero agonist concentration. The concentration of the proghrelin products that suppressed PP release by 50 % of maximum inhibition is referred to as EC_{50}. For each dose-response curve, the EC_{50} and Hill coefficient were calculated by non-linear least square regression analysis, using the statistical package R (34). For display purposes, the various dose-response curves were used to generate a single logistic curve. The mean (± SEM) of the pEC_{50} and of the midpoint slopes of each of the individual curves were calculated. In addition, the pEC_{50} value and the slope were calculated from the curve constructed from the mean values.

2.5.2. Antagonist-induced inhibition of the effect of the agonists: Agonist dose-response curves were constructed without desacyl ghrelin in the medium and in the presence of various concentrations of desacyl ghrelin. The agonist dose-response curves were fitted to the Hill equation. Since increasing doses of the antagonist produced parallel, rightward shifts of the agonist dose-response curves with no change in the lower asymptote, a Schild plot was constructed by plotting the log antagonist concentration on the x-axis against the log (EC_{50} concentration ratio – 1) on the y-axis. Acyl ghrelin concentration ratios were defined as the ratios between EC_{50} concentrations of acyl ghrelin with or without desacyl ghrelin. The plots were fitted by linear regression. The point of intersection with the x-axis gives the concentration of antagonist corresponding to a concentration ratio of 2 (pA_2) (35,36). In a case of typical competitive inhibition the Schild plot should be a straight line with a slope close to unity. The goodness of fit to a straight line was assessed by linear regression analysis and expressed as the square of the correlation coefficient (r^2). For display purposes, the computed parameters were expressed as means and a Schild plot was generated.

3. **Results**

3.1. Effects of obestatin, acyl ghrelin and desacyl ghrelin on spontaneous PP release

Obestatin and acyl ghrelin suppressed the spontaneous PP release in a concentration-dependent manner (Fig 1 A and B). Obestatin had an EC_{50} value ranging from 0.3 to 1.1x 10^{-13} M versus 1.7 to 2.8x10^{-13} M for acyl ghrelin. The midpoint slopes were found to differ from unity, ranging from 0.21 to 0.35 in the case of obestatin and from 0.28 to 0.34 in the case of acyl ghrelin. By contrast, desacyl ghrelin did not affect PP release at any concentration tested (Fig 1 C). Reported plasma levels of the three proghrelin-derived peptides are shown for comparison in Fig.1.The results of the analysis of the concentration-response curves are summarized in Table 1.

3.2. Desacyl ghrelin reverses acyl ghrelin-induced but not obestatin-induced suppression of PP release in a competitive manner.

The effect of obestatin was unaffected by desacyl ghrelin in the incubation medium, even at quite high doses (Fig 2 A). In contrast, the concentration-response curve for acyl ghrelin was greatly affected by desacyl ghrelin, causing the acyl ghrelin dose-response curve to shift to the right in a parallel manner upon addition of increasing concentrations of desacyl ghrelin, in a fashion suggesting competitive inhibition (Fig. 2 B). Desacyl ghrelin did not affect the lower asymptote of the dose-response curves. All acyl ghrelin dose-response curves (normalised data) were fitted to the Hill equation, giving midpoint slopes ranging from 0.30 to 0.45.

3.3. Schild plot analysis

The parallel right-shift of the acyl ghrelin dose-response curves (normalised data) in the presence of increasing concentrations of desacyl ghrelin is illustrated in Fig.2 B, showing the effect of increasing doses of desacyl ghrelin on the acyl ghrelin EC_{50} value. In this experiment the EC_{50} value for acyl ghrelin in the absence of desacyl ghrelin was 2.8 x 10^{-13} M. Analysis of the resultant Schild plot revealed a slope of 0.78 (confidence interval 0.70-0.86) and an intercept on the x axis consistent with an apparent pA_2 value of 14 (Fig.2 C).

4. **Discussion**

The physiological significance of the known proghrelin peptides, i.e. acylated ghrelin, desacyl ghrelin and obestatin, remains to be defined. It cannot be excluded that the different proghrelin peptides act in concert (28) and that their individual as well as their joint effects have to be elucidated before the overall physiological significance of the ghrelin system can be assessed. Indeed, the secretion of all known pancreatic islet hormones are influenced by proghrelin-derived peptides (28) but whether this is physiologically relevant or not remains to be settled. In the present study we made use of the observation that both acylated ghrelin and obestatin exhibited a powerful concentration-dependent suppressive effect on the release of the pancreatic hormone PP.

PP was discovered in 1973 (37,38) and its localization to a specific endocrine cell type in the pancreas was described in 1974-1976 (39-41). The PP release in response to food is under vagal cholinergic control (42-45). PP is known to slow down gastric emptying (46) and to suppress food consumption (45-49). In addition, it has been reported to lower energy expenditure and to reduce expression of the ghrelin gene in the stomach wall (49). The powerful PP release-suppressing effect of acyl ghrelin and obestatin (31) encouraged us to make use of isolated mouse islets as a tool to study how the various proghrelin peptides interact with receptors to acyl ghrelin and obestatin.

4.1. Effects of acyl ghrelin, obestatin and desacyl ghrelin on the spontaneous release of PP.

The present study shows that both obestatin and acyl ghrelin effectively suppressed the spontaneous release of PP from isolated islets of the mouse pancreas, which is consistent with previous findings (31). Desacyl ghrelin was without effect.

It is interesting that the midpoint slope of the dose-response curves for both acyl ghrelin and obestatin, analysed by the Hill equation, differed from unity. The slope for both agonists ranged from 0.2 to 0.4, suggesting negative cooperativity. As we have shown in this paper and in a previous one (31), acyl ghrelin and obestatin suppress PP release, probably by interfering in some unknown way with the sequence of events that is triggered by binding of either of the two agonists to G protein-coupled receptors, i.e. affecting transmembrane ionic (e.g. Ca^{2+}) transport and the intracellular transport, docking and fusion of secretory granules with the cell membrane and/or, finally, affecting the last step of the exocytotic release of PP from membrane-bound secretory granules. Negative cooperativity may reflect excessive receptor rerserve or the existence of more than one class of binding sites (e.g. active versus inactive states of the receptor), or it may reflect interference at some point with the intracellular chain of events that lead to PP release. It should be realized that measurement of the response to an agonist does not always reflect affinity to receptor, especially since we are dealing with isolated islets rather than isolated cells.

4.2. Effect of desacyl ghrelin on the suppression of PP release by acyl ghrelin and obestatin.

Desacyl ghrelin *per se* was without effects on the release of PP. However, our results suggest that, although desacyl ghrelin does not affect PP release, it effectively inhibits the PP release-suppressing effect of acyl ghrelin but not that of obestatin. This observation supports the view that acyl ghrelin and obestatin suppress PP release through separate receptors. There is convincing evidence that that the acyl ghrelin receptor is constitutively active (50,51). Since desacyl ghrelin failed to influence the spontaneous release of PP it seems that desacyl ghrelin does not express any inverse agonist activity. Our results indicate that desacyl ghrelin acts as a competitive and surmountable inhibitor of the PP release-suppressing action of acyl ghrelin.

An antagonist is considered competitive and reversible when it binds to the same receptor site as the agonist and if an equilibrium exists between the agonist and the antagonist so that an increase in the concentration of one decreases the binding of the other. For an antagonist to be competitive the following two criteria have to be met: 1. Similarity of maximum agonist response at different antagonist concentrations. 2. Parallel rightward shift of the agonist dose-response curves (i.e. equality of midpoint slopes) with increasing antagonist concentrations. Desacyl ghrelin reversed the effects of acyl ghrelin in a manner that fulfilled both these criteria.

4.3. Kinetics of desacyl ghrelin-induced inhibition of acyl ghrelin-induced suppression of spontaneous PP release.

If the two critera listed above are met, the pA_2 value can be estimated. The pA_2 value provides an assessment of the power of a competitive antagonist; it is the negative logarithm of the concentration of antagonist that makes it necessary to double the agonist concentration in order to obtain the same effect as in the absence of antagonist. If the slope of the Schild plot is one (or close to one), the pA_2 value is equivalent to the negative logarithm of the dissociation constant of the antagonist with the receptor. However, the analysis of our data revealed that the slope of the Schild plot, being 0.78, differed from unity. The reason why the slope differed from unity is unclear. The apparent pA_2 for desacyl ghrelin was 14, suggesting that the antagonist is quite potent.

4.4. Obestatin and acyl ghrelin receptors.

The present study is concerned with the receptors to obestatin and acyl ghrelin, that suppress the release of PP from pancreatic islets of the mouse.The results indicate that obestatin and acyl ghrelin act on separate receptors. Acylated ghrelin is an endogenous ligand of the growth hormone secretagogue receptor, GHS-R (1). However, there is preliminary evidence that there is more than one receptor for acyl ghrelin in that the sensitivity to acyl ghrelin has been found to differ greatly from one islet cell type to another (31). Indeed, two known GHS-R subtypes are generated by alternative splicing of the receptor gene (52). In addition, acylated ghrelin has been found to act through unidentified receptors in cell lines devoid of GHS-R (53). The orphan receptor G-protein-coupled receptor 39 (GPR39) is the proposed obestatin receptor (21). Experimental observations suggest the existence of more than one form of obestatin receptor with different sensitivities to the ligand (54,55).

4.5. Physiological implications of the findings.

4.*5.1. Implication of the findings with respect to the functional role of acyl ghrelin and obestatin:* Our findings suggest that both acyl ghrelin and obestatin suppress spontaneous PP release. Desacyl ghrelin *per se* does not affect PP release, while acting as a powerful/potent inhibitor of acyl ghrelin but not of obestatin. The physiological significance of our findings remains unknown. Both acyl ghrelin and obestatin circulate in concentrations that may affect the spontaneous release of PP (Fig. 1). However, it may be argued that the three proghrelin-derived peptides are likely to act in concert and that the end result of the concomitant release of the proghrelin-derived peptides (i.e. their effects on PP release from islets) will reflect not only the receptor make-up of the target cells but also the circulating concentration of all three peptides, acyl ghrelin, desacyl ghrelin and obestatin. Plasma desacyl ghrelin levels are typically 5-10- fold higher than acyl ghrelin in rat, mouse and man (desacyl ghrelin levels in rodents ranging between 150-600 pmol/l while acyl ghrelin levels range between 10-50

pmol/l) (8,10). Obestatin levels are also higher than acyl ghrelin levels, ranging between 100-800 pmol/l (9,21,56). The circulating concentrations of acyl ghrelin and desacyl ghrelin are known to vary with the prandial state, being 3-10 times higher in fasted than in fed animals (1,4,7-10). However, the circulating concentrations of obestatin are much less affected by the prandial state (9,21,54). It may be argued that the methods currently in use to measure obestatin and the ghrelin peptides need to be better documented and characterized. Still, we wish to argue that on the basis of their reported circulating concentrations both acyl ghrelin and obestatin should be capable of suppressing PP release in a physiologically relevant manner. In fact, both peptides are reported to circulate in concentrations that can be expected to suppress spontaneous PP secretion by 80 % or more (Fig. 1) Nonetheless, we have to question the physiological significance of circulating acyl ghrelin for the following reasons: 1) Allegedly, desacyl ghrelin circulates in much higher concentrations than acyl ghrelin (see refs 5,8-10), and 2) desacyl ghrelin is a powerful competitive inhibitor of acyl ghrelin (Fig.2, this study). In consequence, the effects of circulating acyl ghrelin can be expected to be severely impaired by desacyl ghrelin (Fig. 3). In contrast, we have no evidence that the effect of obestatin is compromised by any circulating agent.

To summarize, the three proghrelin-derived peptides (acyl ghrelin, desacyl ghrelin and obestatin) exist in the circulation at physiologically relevant concentrations, and circulating desacyl ghrelin is likely to impair the effect of acyl ghrelin. Whether our observations on the PP release-suppressing acyl ghrelin receptor in the pancreatic islets can be extrapolated to acyl ghrelin receptors elsewhere is unknown.

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Legends to Figures

Fig .1
(A, B, C). Concentration-response curves showing the effects of obestatin (A), acyl ghrelin (B) and desacyl ghrelin (C) on the spontaneous release of PP from fresh islets, collected from the duodenal part of mouse pancreas and suspended in 1 ml of a medium enriched with 12 mM glucose. Each incubation vial contained 12 islets per ml medium and the incubation lasted 1 h. The concentration-response curves were constructed from experiments performed during a time span of more than 1 year. Obestatin and acyl ghrelin suppressed the release of PP quite effectively, while desacyl ghrelin was inactive. The release of PP is expressed as amoles per islet and h. The curves shown are from 12 (A), 15 (B) and 8 (C) individual concentration-response curves, each point is the mean value (vertical bars give SEM). The EC_{50} value for acyl ghrelin is 2 $x10^{-13}$ M, the mean midpoint slope is 0.30. The EC_{50} value for obestatin is 10^{-13} M and the mean midpoint slope is 0.35. For a summary see Table 1. Hatched areas in the Fig. show the range of reported blood concentrations of obestatin, acyl ghrelin and desacyl ghrelin in fasted or fed mice and rats (1,4,7-10,21). From the concentration-response curves it seems that the circulating concentrations of both obestatin and acyl ghrelin will cause 80-90 % suppression of spontaneous PP release.

Fig. 2
(A, B). The concentration-response curve for the PP-suppressing effect of acyl ghrelin but not for obestatin was shifted to the right in a parallel fashion in the presence of increasing amounts of desacyl ghrelin in the medium. Each curve is based on normalised data, representing the means of 6-8 individual concentration–response curves for each concentration of desacyl ghrelin: control (filled circle, no desacyl ghrelin added), 10^{-14} M (open circle), 10^{-12} M (filled square), 10^{-10} M (open square) and 10^{-8} M (filled diamond). SEM values are not shown.
(C). A Schild plot was constructed from the data in B, illustrating the competitive antagonism displayed by desacyl ghrelin *versus* acyl ghrelin. An apparent pA_2 value of 14 is indicated by the point of intersection of the line with the x-axis (drawn without constraining the slope to unity). The slope of the Schild plot is 0.78. The square of the correlation coefficient for the Schild plot (r^2) is 0.99.

(Fig. 3).
The cartoon illustrates how proghrelin in the ghrelin (A-like) cells of the stomach gives rise to obestatin and desacyl ghrelin. A proportion of desacyl ghrelin is acylated. The cleavage of proghrelin and the processing of the products is thought to be followed by the parallel secretion of obestatin, desacyl ghrelin and acylated ghrelin. Obestatin and acyl ghrelin suppress the spontaneous secretion of PP (whether the PP cells are the direct target for this effect remains unknown). Desacyl ghrelin *per se* does not affect PP secretion. However, from the results presented in Fig. 2, it seems that it acts as a powerful competitive inhibitor. At circulating concentrations (from 1.5 to 6.0 x 10^{-10} M), it can be expected to greatly impair the effect of circulating concentrations of acyl ghrelin (indicated by X in the cartoon). We suggest that while circulating obestatin is likely to play a physiologically relevant role in suppressing spontaneous PP release, such a role is less likely for acylated ghrelin.

Table 1

Suppression of PP release by obestatin, acyl ghrelin and desacyl ghrelin: pEC_{50} values and midpoint slopes calculated from all available concentration-response curves

Compound	**pEC_{50}**	**mid-point slope**	**n**
obestatin	13.0 ± 0.66[a] 12.9[b] 13.5[c]	0.35 ± 0.05 0.25 0.21	12
acyl ghrelin	12.8 ± 0.31[a] 12.6[b] 12.8[c]	0.30 ± 0.02 0.28 0.34	15
desacyl ghrelin	no effect	no effect	8

[a] pEC_{50} values and mid-point slopes were calculated from the individual dose-response curves (raw data) followed by calculations of means and SEM. [b] pEC_{50} and mid-point slopes were calculated from the curves constructed using mean normalized data. [c] pEC_{50} and mid-point slopes were calculated from the curve constructed using mean raw data data. n is the number of individual dose-response curves. For definition of the various parameters see Methods.

Figure 1

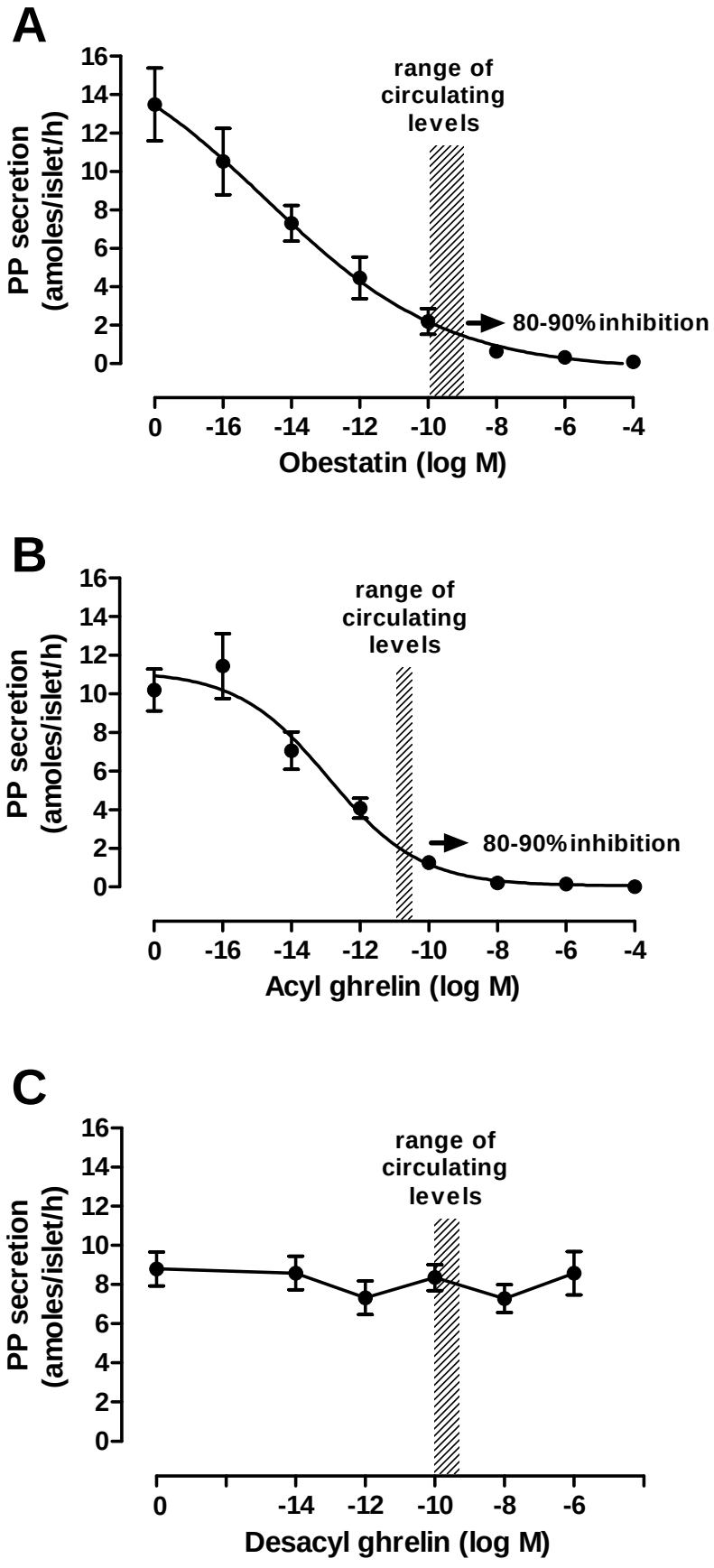

A
PP secretion (amoles/islet/h)
range of circulating levels
80-90% inhibition
Obestatin (log M)
B
PP secretion (amoles/islet/h)
range of circulating levels
80-90% inhibition
Acyl ghrelin (log M)
C
PP secretion (amoles/islet/h)
range of circulating levels
Desacyl ghrelin (log M)

Figure 2

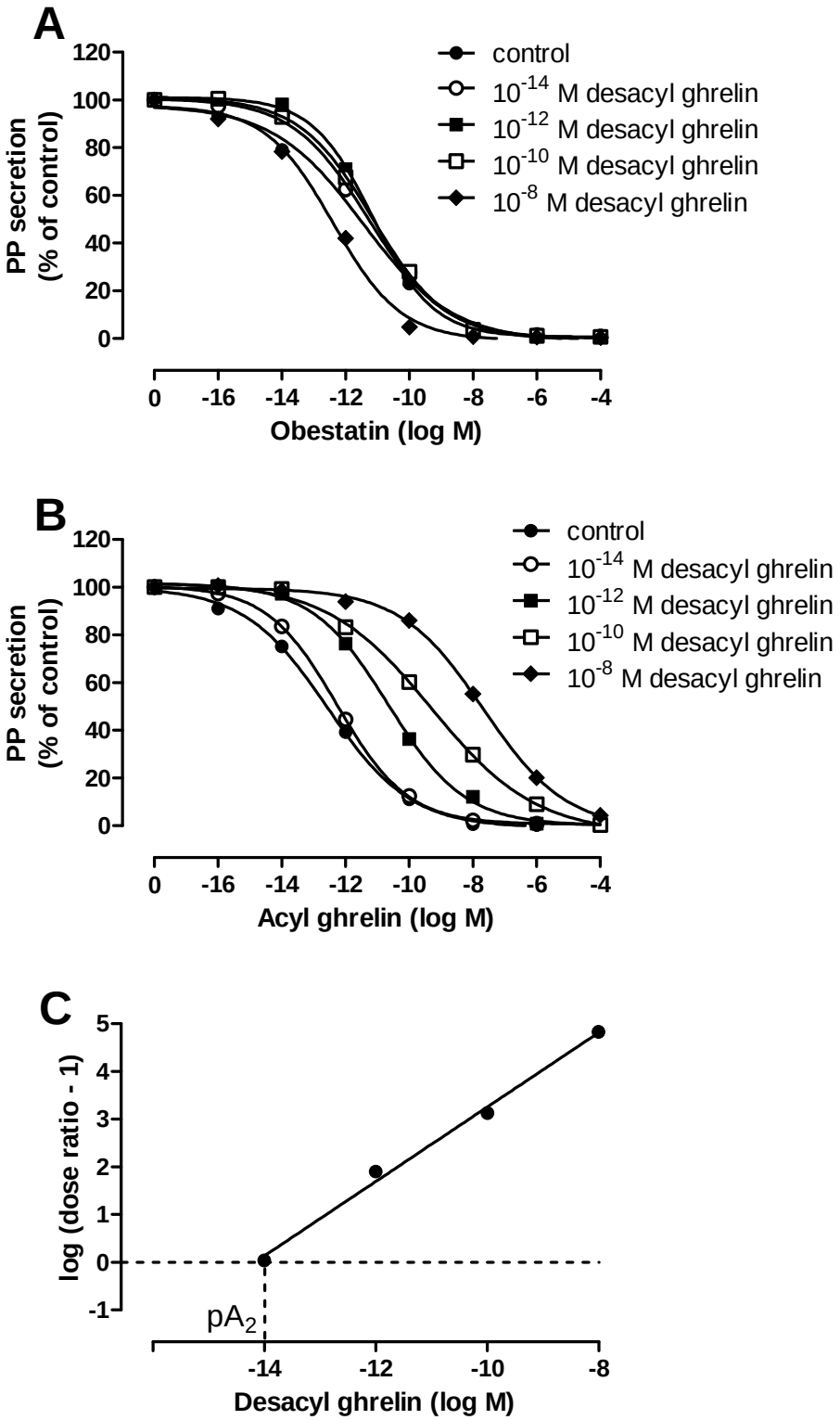
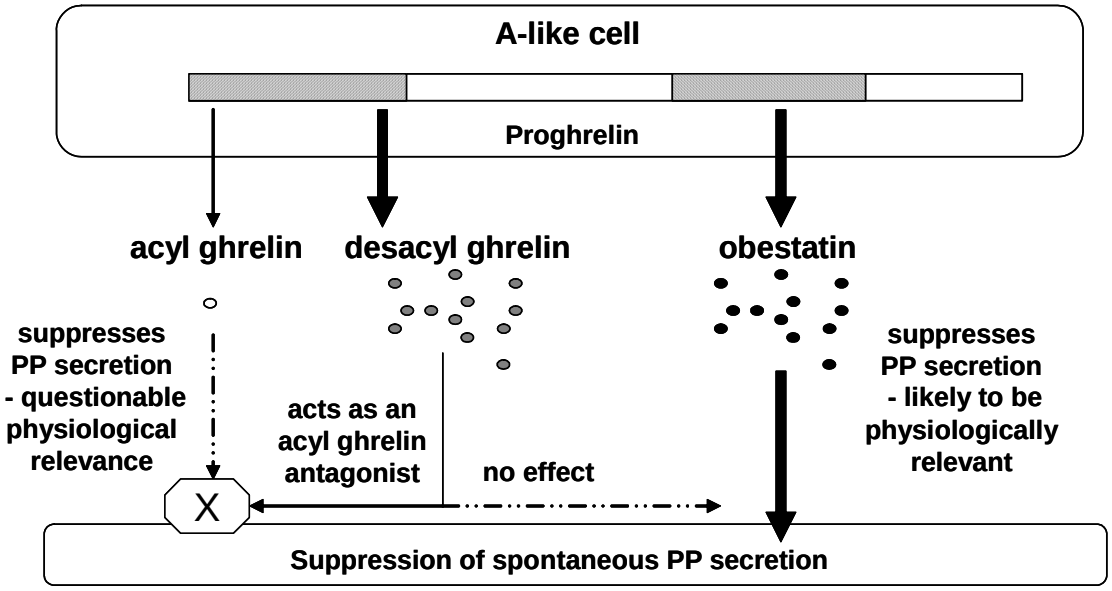

A
PP secretion (% of control)
120
100
80
60
40
20
0
control
10-14 M desacyl ghrelin
10-12 M desacyl ghrelin
10-10 M desacyl ghrelin
10-8 M desacyl ghrelin
0
-16
-14
-12
-10
-8
-6
-4
Obestatin (log M)
B
PP secretion (% of control)
120
100
80
60
40
20
0
control
10-14 M desacyl ghrelin
10-12 M desacyl ghrelin
10-10 M desacyl ghrelin
10-8 M desacyl ghrelin
0
-16
-14
-12
-10
-8
-6
-4
Acyl ghrelin (log M)
C
log (dose ratio - 1)
5
4
3
2
1
0
-1
pA2
-14
-12
-10
-8
Desacyl ghrelin (log M)

Figure 3


A-like cell
Proghrelin
acyl ghrelin
desacyl ghrelin
obestatin
suppresses PP secretion - questionable physiological relevance
acts as an acyl ghrelin antagonist
no effect
suppresses PP secretion - likely to be physiologically relevant
X
Suppression of spontaneous PP secretion