RAPID COMMUNICATION

Growth hormone and somatostatin directly inhibit gastric ghrelin secretion. An *in vitro* organ culture system

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ABSTRACT. Ghrelin is a 28-amino-acid hormone produced mainly by the stomach which strongly promotes food intake. It is the only known peripheral orexigenic hormone that induces the release of GH. Ghrelin has been proposed as a link between the enteric system and central regulation of energy balance and growth. Although it has recently been the focus of extensive study, the secretion mechanism is not yet well characterized. The aim of this study was to test the direct effect of hormones from the somatotropic axis on ghrelin release directly from the stomach. To this end, an organ culture model of gastric tissue explants from rat donors was used. These stomach explants were incubated in 6 well plates for 1, 2, and 3 h after treatment with either GH, GHRH, SS or IGF-I, all them at 10^{-6} M. After incubation, the medium was collected and the amount of ghrelin secreted by the gastric tissue was measured by radioimmunoassay. It was observed that GH and SS significantly decreased gastric ghrelin secretion, while GHRH and IGF-I had no effect on the present model. These results would confirm the capacity of GH and SS to act directly upon gastric level, inhibiting ghrelin secretion *in vitro*.

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INTRODUCTION

The main function of the somatotropic axis is the regulation of GH secretion and general metabolism (1). The somatotropic axis is made up of GH, GHRH, somatostatin (SS), and IGF-I, and the recently incorporated ghrelin. This 28-amino-acid peptide (2) is expressed in a large number of tissues (3-5) but mainly in endocrine cells within the oxyntic gland of the stomach (6).

There is evidence suggesting a relation between ghrelin and the other components of the somatotropic axis (7, 8). GH is controlled by metabolic status and is profoundly altered in states such as obesity, malnutrition, fasting, and diabetes mellitus (1). For its part, plasma ghrelin is also affected by nutritional status, being enhanced in the fasting state and reduced in obesity and situations of positive energy balance (9-11). In light of this, ghrelin may well be the link between the somatotropic axis and metabolism.

There is currently controversy over the effect of GH on the regulation of ghrelin secretion. It is difficult to explain as the interplay between GHRH, GH, and ghrelin levels has not yet been established. Exogenous treatment with another component of the somatotropic axis SS and its analogues has been reported to produce inhibition of plasma ghrelin levels in normal subjects (12). Systemic administration of SS suppresses the secretion of a wide range of splanchnic hormones, such as insulin, glucagon, and gastroentero-pancreatic hormones and ghrelin may be part of that list.

IGF-I is a primary mediator of GH functions as a negative feed-back regulator of GH secretion *in vivo*. However, little is known about how IGF-I affects the regulation of ghrelin secretion. A negative correlation between these two hormones has been reported in humans (13), and there have been reports of a down regulation of IGF-I on ghrelin receptor (GHS-R) in rat pituitary (14).

Although it is commonly assumed that any variation of plasma ghrelin reflects changes in the rate of secretion by the stomach, this has not been looked into. The intimate mechanisms governing the biosynthesis and secretion of

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stomach-derived ghrelin are unknown. The purpose of the present study was to evaluate the interaction between the components of the somatotropic axes such as GH, GHRH, SS, and IGF-I and gastric ghrelin released directly by the stomach without interferences from other organs. To do so, a model of gastric tissue explant cultures validated by our group was used.

MATERIALS AND METHODS

Animals and drugs

Adult Sprague Dawley rats were used for all experiments. Experimental animals were housed in 12 h light/12 h dark cycles with free access to food and water (no.=10). Research was conducted according to protocols approved by the Animal Care Committee of Santiago de Compostela University.

SS and IGF-I were purchased from Sigma Chemical Co. (St Louis, MO, USA), GHRH was obtained from Serono (Madrid, Spain), GH was supplied by Pfizer (Sant Cugat del Vallés, Barcelona).

Tissue-explant cultures

Gastric explant cultures were performed as previously described (15). In brief, immediately after euthanasia, the stomachs were rapidly excised and transported to the incubator in sterile Krebs-Ringer-Hepes buffer. After blood vessels and connective tissue were removed, stomach tissue was washed. Approximately 1 g of tissue, mostly gastric fundus, was then placed in each of 6 well dishes containing 2.5 ml of Dulbecco's modified Eagle's medium supplemented with penicillin (100 U/ml) and streptomycin sulphate (100 µg/ml) and incubated at 37 C under a humidified atmosphere of 95% air-5% CO_2. After a pre-incubation period of 1 h, the media was discarded and 2.5 ml of fresh medium was dispensed into each well. Culture medium was finally collected at 1, 2 or 3 h and tissue was weighed with a precision scale. Media was stored at –20 C until ghrelin assay.

Biochemical analysis

Total ghrelin levels were determined by means of a double antibody radioimmunoassay (RIA) using reagents kits and methods provided by Phoenix Pharmaceuticals Inc. (Belmont, CA), as previously described (16). The limit of assay sensitivity was 1 pg/ml. Results were expressed as pg/ml of ghrelin per g of tissue in culture media. Data is expressed as mean±SE and assessed by the Mann-Whitney test. $p<0.05$ was considered significant.

RESULTS

In order to assess the action of the hormones making up the somatotropic axis on gastric ghrelin secretion *in vitro*, several kinds of treatment were carried out. Solutions of 10^{-6} M of GH, GHRH, SS or IGF-I or vehicle were added to the culture plate incubation medium.

The treatment of gastric tissue explants with GHRH 10^{-6} M did not affect gastric ghrelin secretion at any time tested (GHRH 10^{-6} M: 1671±84 pg/ml per g of tissue *vs* control: 1698±34 pg/ml/g of tissue at 2 h of incubation) (Fig. 1).

Similarly, IGF-I treatment also had no effect on gastric ghrelin secretion (IGF-I 10^{-6} M: 1686 pg/ml/ per g of tissue *vs* control 1511 pg/ml/per g of tissue) (Fig. 1).

In contrast, SS treatment significantly decreased basal ghrelin secretion from tissue explants at 2 and 3 h of incubation (SS 10^{-6} M: 1963±89 pg/ml/per g of tissue *vs* 1814±100 pg/ml/per g of tissue control at 1 h; 1381±61 pg/ml/per g of tissue *vs* 1698±34 pg/ml/g of tissue control, $p<0.01$; 1415±221 pg/ml/g of tissue *vs* 1959±63 pg/ml/g of tissue control at 3 h of incubation, $p<0.05$) (Fig. 1). The same occurred regarding treatment with GH (GH 10^{-6} M: 1512±54 pg/ml/per g of tissue *vs* 1814±100 pg/ml/per g of tissue control at 1 h; 1184±122 pg/ml/per g of tissue *vs*

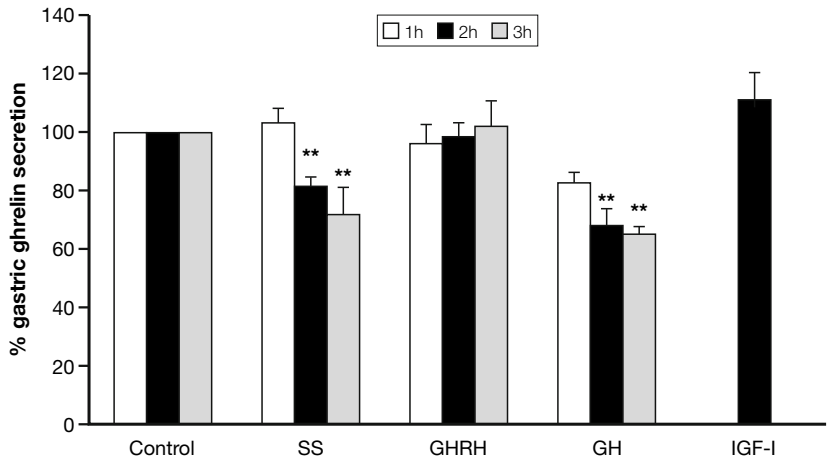

Fig. 1 - *Ghrelin secretion directly from gastric tissue explants presented as % over control (mean±SE) after incubation for 1, 2 and 3 h of SS, GHRH, GH, and IGF-I at doses of 1 µM. Samples were measured by duplicate, no.=10. (**p<0.01 vs control).*

control: 1698±34 pg/ml/per g of tissue at 2 h of incubation, $p<0.01$; 1280±51 pg/ml/per g of tissue *vs* 1960±9 pg/ml/per g of tissue control at 3 h, $p<0.01$) (Fig. 1).

DISCUSSION

The present study was conducted using an organ culture model of gastric tissue capable of assessing the direct regulation of ghrelin secretion by the stomach (15). With this model, a 2-3 h incubation with GH and SS was found to significantly decrease ghrelin secretion directly from the stomach *in vitro*, thus suggesting for the first time that the observed plasma ghrelin changes induced by these hormones would be directly due to changes in gastric ghrelin release. On the other hand, both GHRH and IGF-I failed to induce modifications on basal gastric ghrelin secretion.

The stimulating effect of ghrelin administration on GH secretion (2, 6, 7) has been widely reported. This, together with the fact that the receptor for ghrelin (GHS-R 1a) is expressed in the pituitary and that the main source of ghrelin is the stomach, suggests that a stomach-pituitary-GH axis may exist. The aim of the present study was to determine whether the classical components of the somatotropic axes could, in turn, be acting at the gastric level to modulate ghrelin secretion.

GHRH, the main hypothalamic stimulatory peptide from the somatotropic axis, has been directly related to ghrelin since the co-administration of GHRH and ghrelin produces a synergistic effect on GH secretion (17). In the present study, it was found that the treatment of gastric tissue explants with a dose of 10^{-6} M of GHRH had no effect on gastric ghrelin secretion from the stomach. This absence of the effect is supported by the fact that GHRH-R expression has never been reported in the stomach. Recently, the expression of a splice variant of GHRH-R, called SV1, has been described at the gastric level (18). However the results here presented seem to indicate that SV1-mediated GHRH actions may include other effects, but not ghrelin secretion.

SS, the inhibitory hypothalamic peptide, is produced locally at the gastric level and suppresses secretion of several gastrointestinal peptides in a paracrine fashion. It has been demonstrated that some SS-producing cells make direct cellular contact with ghrelin-producing cells in the gastric fundus (19, 20), suggesting a regulatory interaction. It was found that treatment with 10^{-6} M of SS induced a clear decrease of gastric ghrelin secretion. This would be the first demonstration that the previously reported *in vivo* reduction of plasma ghrelin by SS might reflect a direct action of ghrelin on the stomach. Expression of SS receptors in the gastric mucosa has been reported (21), which suggests that gastric ghrelin release may be modulated by a direct activation of gastric SS receptors.

Several data in the literature suggest a negative GH feedback on stomach ghrelin (22). This is supported by the fact that GH receptors are present in the stomach and intestine (23). However a review of the bibliography reveals an apparent contradiction regarding the effect of GH levels on ghrelin (24, 25). The findings in the present study showing that treatment with a dose of 10^{-6} M of GH directly on gastric tissue explants induced an inhibitory effect on ghrelin release from the stomach should shed some light on this dilemma. The indication that variations in circulating ghrelin induced by changes in circulating GH levels are due to a direct action of GH on the stomach would be the first evidence of a direct GH effect on gastric ghrelin secretion.

The mechanism of action behind the GH-mediated reduction of ghrelin levels may involve the release of gastric SS produced locally in gastric tissue. The increase in GH levels could be activating gastric GH receptors and up-regulating SS tone in the stomach in order to reduce ghrelin release as well as consequent ghrelin plasma levels.

IGF-I is a primary mediator of GH functions as a negative feedback regulator of GH secretion and has an inhibitory action directly on the pituitary expression of GHS-R 1a (14). At gastrointestinal level, a positive expression of IGF-I as well as IGF-I receptor has been described (26), which may play a role in the regeneration of intestinal mucose (27). The relation between IGF-I and gastric ghrelin regulation has not yet been definitively elucidated. In fact, some controversy exists as some authors report a negative correlation while others report no correlation at all (13). In the present study, a positive effect of IGF-I directly on stomach regulation has been discarded.

In conclusion, to the best of our knowledge this result might constitute the first demonstration that both GH and SS reduce plasmatic ghrelin by a direct inhibitory action on the stomach. GHRH and IGF-I were devoid of action in this model.

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