RAPID COMMUNICATION

Ghrelin elicits a marked stimulatory effect on GH secretion in freely-moving rats

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Abstract

Ghrelin is a growth hormone-releasing acylated peptide from stomach. The purified peptide consist of 28 amino acids in which the serine 3 residue is n-octanoylated. Ghrelin has been reported to increase *in vitro* GH secretion as well as *in vivo* plasma GH levels in pentobarbital anaesthetized rats. The aim of this work was to characterize the stimulatory effect of Ghrelin on *in vivo* GH secretion in freely-moving rats. Furthermore, we compare the effect of Ghrelin with GHRH.

In addition to vehicle, we administered different doses of Ghrelin (3 nmol/Kg, 12 nmol/Kg and 60 nmol/Kg); GHRH (3 nmol/Kg and 12 nmol/kg). Plasma GH levels were measured in blood samples taken at 5, 10, 15, 20, 30 and 45 min after their administration as an i.v. bolus at 0 min.

Administration of Ghrelin led to an increase in plasma GH levels at all time-points tested (5, 10, 15, 20 and 30 min, $P < 0.01$; and 45 min, $P < 0.05$) in comparison to control untreated rats. A maximal stimulatory effect on plasma GH was observed following administration of 12 nmol/Kg of Ghrelin, the effect being similar to the one obtained with 60 nmol/Kg in terms of both AUC and mean peak GH levels. At the dose of 3 nmol/Kg GHRH and Ghrelin exhibited a similar stimulatory effect in term of both, AUC and mean peak GH levels. However following administration of a dose of 12 nmol/Kg, the effect of Ghrelin was much greater than the same dose of GHRH in terms of both AUC and mean peak GH levels.

In summary, this study provides the first evidences that Ghrelin exert a marked stimulatory effect in plasma GH levels in freely-moving rats and provides further evidences that Ghrelin may play an important role in the physiological control of GH secretion.

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Introduction

Growth hormone secretagogues (GHSs) are artificial compounds which release GH in all species tested so far. Up to now, these molecules mimicked an unknown endogenous factor that activates the GHS-receptor (1–3). The earlier cloning of GHS-R suggested that an endogenous ligand for this receptor might exist (4). Indeed, after intensive searches by different groups, the isolation of an endogenous ligand of the GHS-receptor, named Ghrelin (5) was recently reported. The purified ligand was found to be a peptide of 28 amino acids, in which the serine 3 residue, was n-octanoylated. This peptide have been shown to exert a very potent and specific GH-releasing activity *in vitro* as well as *in vivo* in pentobarbital anaesthetized rats. Taking into account that it is secreted prevalently from the stomach and that Ghrelin circulates in normal subjects at considerable plasma concentrations it has been postulated that this molecule is secreted from the stomach, circulating in the blood stream to stimulates GH secretion by the somatotrophs (5). More recently a second endogenous

ligand for the GHS-R named des-Gln14-ghrelin, has been purified and characterized (6). Its biological activity and sequence is identical to Ghrelin except for one glutamine in position 14.

It is well known that *in vivo* GH secretion is pulsatile. The male rat exhibit a pattern of GH secretion consisting of pulses of high amplitude, on average one every three hours, with almost undetectable troughs in between (7, 8). The aim of this paper was to assess the stimulatory of Ghrelin on *in vivo* GH secretion in the rat. To this end we have used freely-moving rats since in this experimental model the physiological regulatory mechanisms are fully operating. Furthermore, we assessed in the same experiments the stimulatory effect of GHRH which is considered to play a dominant regulatory role on GH synthesis and secretion.

Materials and methods

Adult male Sprague–Dawley rats (200–250 g) were kept with a ratio of 12 h light:12 h darkness in a temperature- and humidity-controlled room. Chronic

Figure 1 Mean $\pm$ S.E.M. plasma GH levels after the administration (i.v.) of vehicle (control), Ghrelin and GHRH in adult male freely-moving rats ($n = 7-17$ rats/group).

intracardiac cannulae were implanted under ketamine-xylazine anaesthesia, as previously described (9). After surgery, the animals were placed directly in isolation test chambers for 5 days and were given free access to regular Purina rat chow and tap water. Thereafter the animals were allowed to feed *ad libitum*. On the day of the experiment, blood samples (0.3 ml) were withdrawn at the appropriate times. The animals ($n = 6-10$ rats/group) received either vehicle, GHRH (Geref, Serono, Spain) or Ghrelin (Peninsula, U.K.) as an i.v. bolus.

Plasma GH concentrations were determined by double antibody RIA using materials supplied by the NHPP as described previously (10). Values are expressed in terms of the GH reference preparation (GH-RP-2). The intra- and interassay coefficients of variation were 2.4% and 4.8% respectively.

Data are expressed as mean $\pm$ s.e.m. Comparison between the different groups was assessed by the Mann-Whitney test.

Results

Administration of Ghrelin led to an increase in plasma GH levels at all time-point tested (5, 10, 15, 20 and 30 min, $P < 0.01$; and 45 min, $P < 0.05$) in comparison to control untreated rats. A maximal stimulatory effect on plasma GH was observed following administration of 12 nmol/Kg of Ghrelin, the effect being similar to the one obtained with 60 nmol/Kg in terms of both AUC and mean peak GH levels (Figs 1 and 2).

Administration of GHRH, either 3 nmol/kg or 12 nmol/Kg also led to a clear increase in plasma GH levels, at 5, 10, 15, 20 and 30 min ($P < 0.01$) in comparison to control rats. At the dose of 3 nmol/Kg GHRH and Ghrelin exhibited a similar stimulatory effect in term of both AUC and mean peak GH levels. However following administration of a dose of

Figure 2 Area under curve (AUC) and mean peak GH levels following the administration of vehicle (control), Ghrelin and GHRH in adult male freely-moving rats. * $P < 0.05$, ** $P < 0.01$, # $P < 0.01$ (control rats versus rats treated with any dose of Ghrelin or GHRH).

12 nmol/Kg, the effect of Ghrelin was much greater than the same dose of GHRH in terms of both AUC and mean peak GH levels.

Discussion

The isolation of Ghrelin is one of the most important breakthroughs in the understanding of the regulatory mechanisms involved in the neuroregulation of GH secretion for several reasons (11). It gives definitive proof of the existence of a GHS-GHS-receptor signaling system in the control of GH secretion. Although for many years it became dogma that GH secretion by the anterior pituitary gland was the net result of the antagonistic actions of GHRH and somatostatin, a new physiological model of the regulation of GH secretion involving GHRH, somatostatin and Ghrelin must now be developed. It opens up the possibility of gaining a greater insight into the physiopathological mechanisms involved in the alterations of somatotroph cell function and somatic growth. Finally, it will allow the development of new agonist and antagonist compounds that may well be useful in the treatment of different disease states.

Previous data have shown that Ghrelin is a potent GH secretagogue both *in vivo* and *in vitro* (5). However, the *in vivo* GH-releasing activity of Ghrelin was tested in pentobarbital-anaesthetized rats, and this experimental model is limited by the fact that pentobarbital inhibits hypothalamic somatostatin release. In the present study we therefore decide to assess the effect of Ghrelin in freely-moving rats, a model that allows all the regulatory mechanisms to be in operation (7, 8).

We found that Ghrelin elicits a clear-cut increase in plasma GH levels at all the doses tested. At the lowest dose tested (3 nmol/Kg) it exhibited a similar GH releasing activity than GHRH. However, the maximal stimulatory effect exerted by Ghrelin was 2 to 3 times greater than GHRH in terms of both AUC and mean peak GH levels. This is a very interesting finding since it shows that *in vivo* Ghrelin is a more potent GH releaser than GHRH, which is in contrast to the their *in vitro* effects where the opposite was described (5). Nevertheless, it should be noted that it is well established that GHRH is needed for somatotroph cell proliferation, and that GH synthesis and GH secretion elicited by most GH stimuli, including synthetic compounds that activate the GHS-receptor, are absent after administration of a GHRH-antagonist as well as after passive immunization with anti-GHRH antiserum (12, 13). Although, direct evidences is lacking at present, it could be postulated that while GHRH may well play a pleiotropic role on somatotroph cell function, GH secretion will be dependent on the antagonistic effects exerted by Ghrelin and somatostatin. Future studies assessing Ghrelin, GHRH and somatostatin levels in portal blood vessels and their relationship to GH pulses should answer this question.

In summary, this study provides the first evidences that Ghrelin exert a marked stimulatory effect in plasma GH levels in freely-moving rats and provides further evidences that Ghrelin may play an important role in the physiological control of GH secretion.

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