ABSTRACT. Background: Administration of ghrelin to women stimulates the secretion of PRL but the mechanism is not known. Aim: The aim of the study was to investigate the effect of the dopamine receptor blocker, metoclopramide, on ghrelin-induced PRL release. Subjects and methods: Ten healthy normally cycling women were studied in the midluteal phase of 4 menstrual cycles. A single dose of normal saline (cycle 1), ghrelin (1 μg/kg) (cycle 2), metoclopramide (20 mg) (cycle 3), and ghrelin plus metoclopramide (cycle 4) was given to the women iv. Blood samples in relation to the iv injection (time 0) were taken at –15, 0, 15, 30, 45, 60, 75, 90, and 120 min. The response of PRL and GH was assessed. Results: Following ghrelin administration (cycles 2 and 4), plasma ghrelin and serum PRL and GH levels increased rapidly, peaking at 30 min (p<0.001). PRL was also increased after the injection of metoclopramide (p<0.001, cycle 3), but the increase was much greater than after the administration of ghrelin. The combination of ghrelin and metoclopramide stimulated PRL secretion to the same extent with metoclopramide alone. No changes in GH and PRL levels were seen after saline injection. Conclusions: These results demonstrate that the stimulating effect of ghrelin on PRL secretion is not additive with that of metoclopramide, although a dose range study might provide further information.

INTRODUCTION

Ghrelin, a peptide hormone of 28 amino acids, is the endogenous ligand of GH secretagogue receptor (GHS-R) (1). This protein is secreted from the mucosa of the stomach and controls appetite and food intake (2). Exogenous administration of ghrelin results in a marked stimulation of GH secretion in men and women (3-7). Apart from GH, ghrelin stimulates the secretion of other pituitary hormones, including PRL (8-10). The effect of ghrelin on GH and PRL secretion has been studied in various normal and abnormal conditions, such as in obese patients (11), women with the polycystic ovary syndrome (10, 12), women with anorexia and bulimia nervosa (13, 14) or in postmenopausal women treated with exogenous estrogen (5, 15). The secretion of GH and PRL in response to an acute iv injection of ghrelin has been also investigated in a recent study in normal women with no differences between the early follicular, the late follicular, and the midluteal phase of the menstrual cycle (7). The mechanism of the stimulating effect of ghrelin on PRL secretion is not clear, although a direct action on the pituitary is likely since this peptide can increase the PRL release from dispersed human fetal pituitaries of midgestation and from cultured GH- and PRL-secreting adenomas (16). It is known that PRL secretion is under the negative hypothalamic control via the action of dopamine. Recently, we have demonstrated that a dopamine agonist, bromocriptine, can block the stimulating effect of ghrelin on PRL secretion (17). Nevertheless, it is not known whether the opposite, i.e. the reduction of dopamine activity would affect ghrelin-induced PRL secretion. The present study was undertaken to investigate the effect of metoclopramide, a dopamine antagonist, on the stimulating effect of ghrelin on PRL secretion in normal women.

MATERIALS AND METHODS

Subjects

Ten healthy normally cycling women aged 20-32 yr (mean 24.9 yr) volunteered for the study and gave written informed consent. Institutional Review Board approval of the study was obtained. The women had a normal body mass index (range 16.5-23.5, mean 19.8 kg/m²). They had not used any type of hormonal or any other medical treatments for at least the last 6 months before entering the study. A single iv injection was given to all women in the midluteal phase of four consecutive spontaneous cycles, i.e. 7 days following ovulation assessed by ultrasonic examination. In the 1st cycle (cycle 1), the women were given normal saline (2 ml), in the 2nd cycle (cycle 2) ghrelin (1 μg/kg) (Clinalfa, Merck Biosciences AG, Laufelfingen, Switzerland), in the 3rd cycle (cycle 3) metoclopramide (20 mg) (injectable solution 10 mg/2ml/amp, Synthelabopharm, Athens, Greece), and in the 4th cycle (cycle 4) ghrelin followed immediately by metoclopramide in the above doses. Blood samples, in relation to the injection of saline or drugs (time 0), were taken from all women at –15, 0, 15, 30, 45, 60, 75, 90, and 120 min. All experiments were performed, after overnight fasting, in the morning (10:00-12:00 h), so that blood PRL had returned to normal levels after its nocturnal increased secretion. The experiments were performed in the midluteal phase because the women were also involved in acute experiments in the early fol-
icular phase of the same cycles, in which ghrelin, GnRH or both drugs were injected (6).
All blood samples were centrifuged at 1000 g for 15 min and plasma and serum were stored at –20°C until assayed. Plasma total ghrelin and serum PRL and GH were measured in all blood samples, while serum FSH, LH, estradiol, and progesterone were measured only at the –15 time point. All hormones were measured in duplicate in each blood sample.

Assays
Measurement of total ghrelin in plasma was performed with the use of a radioimmunoassay (KIPMR90, BioSource Europe S.A., Nivelles, Belgium). The results are expressed as pg/ml. GH, PRL, FSH, and LH were measured in serum using an immunoradiometric assay (hGH-IRMA, PRL-IRMA, FSH-IRMA, and LH-IRMA respectively, BioSource Europe S.A.). The results are expressed as pg/ml and ng/ml respectively. The lower limits of detection for ghrelin, GH, PRL, LH, estradiol, and progesterone were 40 pg/ml, 0.07 ng/ml, 0.35 ng/ml, 0.1 mIU/ml, 0.2 mIU/ml, 2 pg/ml, and 0.05 ng/ml respectively. Inter- and intra-assay coefficients of variation were 7.3 and 5.0%, 6.7 and 0.7%, 4.5 and 3.3%, 2.4 and 1.1%, 3.4 and 1.4%, 6.2 and 4.9% and 6.5 and 3.3%, respectively.

Data analysis
Hormone values were normally distributed (one sample Kolmogorov-Smirnov test) and statistical analysis was performed by repeated measures one-way analysis of variance (ANOVA) and post-hoc testing. An α-level of 0.05 was used to determine statistical significance. All values are expressed as mean±SEM. The statistical software package used was NCSS 2001 (Number Cruncher Statistical Systems, Kaysville, UT, USA).

RESULTS
Hormonal characteristics of the women before the injection of the drugs in the 4 cycles are shown in Table 1. Hormone values were compatible with the stage of the cycle, i.e. the women ovulated in all 4 cycles. The duration of all cycles ranged between 27 and 30 days. Plasma ghrelin levels increased rapidly following its injection (cycles 2 and 4), reaching a peak at 30 min and declining gradually thereafter (p<0.001) (Fig. 1). Ghrelin levels declined gradually thereafter until the point of 120 min. There were no significant differences in ghrelin levels between cycles 2 and 4 at all time points. In cycles 1 and 3, plasma ghrelin concentrations remained unchanged during the whole period of the experiment (Fig. 1). From the point of 15 min to the point of 120 min, plasma ghrelin levels were significantly higher in cycles 2 and 4 than in cycles 1 and 3 (Fig. 1).

Figure 2 shows serum PRL and GH levels before and after the injection of drugs. Following the injection of ghrelin alone (cycle 2), serum PRL levels increased significantly reaching a peak at 30 min and decreasing gradually thereafter (p<0.001) (Fig. 2A). Metoclopramide administration (cycle 3), induced a marked increase in serum PRL levels that reached a peak at 15 min and declined gradually thereafter (p<0.001) (Fig. 2A). The increase of PRL levels was greater following metoclopramide than ghrelin injection (~15-fold vs ~3-fold over baseline). When ghrelin was injected simultaneously with metoclopramide (cycle 4), PRL levels increased similarly to metoclopramide alone with no significant differences in PRL levels between cycles 3 and 4 at all time points (Fig. 2A). The injection of normal saline (cycle 1) had no effect on serum PRL levels. PRL values were significantly higher in cycles 3 and 4 than in cycles 1 and 2 from the point of 15 min to the point of 120 min (p<0.05). Also, in cycle 2, serum PRL levels were significantly higher than in cycle 1 from the point of 15 min to the point of 60 min (Fig. 2A).

The secretion of GH was significantly stimulated by the injection of ghrelin (cycles 2 and 4). Following the injection, serum GH concentrations showed a rapid increase, peaking at the point of 30 min and declining gradually thereafter (p<0.001) (Fig. 2B). There were no significant differences in serum GH levels between cycles 2 and 4 at any time point. The injection of metoclopramide alone (cycle 3) did not affect serum GH levels that remained stable throughout the whole experimental period. Similarly, following the injection of normal saline (cycle 1), serum GH levels remained unchanged. The levels of GH were significantly higher in cycles 2 and 4 than in cycles 1 and 3 from the point of 15 min to the point of 90 min (Fig. 2B).

Table 1 - Hormonal characteristics of the women (mean±SEM).

<table>
<thead>
<tr>
<th>Cycle 1</th>
<th>Cycle 2</th>
<th>Cycle 3</th>
<th>Cycle 4</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol (pg/ml)</td>
<td>115±21.2</td>
<td>105±28.1</td>
<td>132±12.2</td>
<td>135±19.4</td>
</tr>
<tr>
<td>Progesterone (ng/ml)</td>
<td>12.2±3.2</td>
<td>15.0±2.5</td>
<td>18.8±2.8</td>
<td>16.1±1.9</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>2.2±0.2</td>
<td>2.9±0.3</td>
<td>3.1±0.4</td>
<td>2.6±0.3</td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
<td>3.1±0.7</td>
<td>3.1±0.6</td>
<td>3.6±0.8</td>
<td>3.9±0.2</td>
</tr>
</tbody>
</table>