The effect of oxytocin administration upon the pulsatile secretion of luteinizing hormone in humans

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Abstract. The effect of iv administration of synthetic oxytocin upon the pulsatile pattern of LH secretion was studied in 5 healthy men and 10 healthy women. Five of the women were studied in the follicular phase of a menstrual cycle and the other 5 were studied in the luteal phase of a cycle. Synthetic oxytocin in 0.9% saline or saline alone was administered via continuous iv infusion for 8 h on 2 consecutive days. Infusions were administered using a double-blinded and randomized schedule. The rate of the oxytocin infusion commenced at 1 mU/min and was increased 1 mU/min every 40 min to a final rate of 12 mU/min. The plasma oxytocin concentration during oxytocin infusion ranged from 2–70 fmol/l. Blood for LH determination was sampled every 20 min in the 5 follicular phase women and every 10 min in the 5 men and 5 luteal phase women. The detect algorithm was used to analyze LH pulsatile secretion. Oxytocin infusion was without significant effect on mean LH, number of LH pulses, or area under the LH curve in men or women studied for the period of observation. Thus it is unlikely that increases in plasma oxytocin regulate the pulsatile secretion of LH in humans.

Oxytocin (OT) is a hormone that is synthesized in magnocellular neurons of the paraventricular nucleus (PVN) and transported to the posterior pituitary gland for storage prior to its release into the circulation. The only clearly established physiological role for OT is to stimulate milk ejection in post partum, lactating women and animals (1). However, OT has been postulated to have a role in other reproductive-related events (2–7) and may interact with the hormones regulating reproduction in mammals, namely steroids and gonadotropins (8,9). OT was found to stimulate LH, and to a lesser degree, FSH release from cultured rat anterior pituitary cells (9) and the intravenous administration of an OT antagonist in the rat decreased the LH surge on the afternoon of proestrus (10). OT has also been found to have a modulatory influence upon GnRH release in vitro. OT inhibited, in a dose-dependent manner, the basal and potassium-stimulated release of GnRH from mediobasal hypothalamic and median eminence explants of rats (11). Although an early study in humans (12) found that administration of synthetic OT increased serum FSH, subsequent studies found no effect of OT upon the basal or GnRH-stimulated release of LH or FSH (13–16). To the best of our knowledge the effect of administration of synthetic OT upon the pulsatile secretion of LH has not been studied in humans. The present study was done to determine if peripheral administration of OT alters the pulsatile pattern of LH secretion in healthy men and normally cycling women.

Subjects and Methods

Volunteers for the study were 5 healthy men and 10 healthy women between the ages of 18 and 35 years who were within 5% of ideal body weight. None of the patients...
had taken medications for at least one month before the study. The women described regular menstrual cycles of normal duration (26 to 30 days). Five women were studied in the early follicular phase of a cycle (days 4–6), and 5 additional women were studied in the early luteal phase of a cycle (days 20–24). Individuals were fasted after midnight; they refrained from caffeine and nicotine for the duration of the study and for 8 h prior to the start of the study. Studies were performed in the Clinical Research Center of the University of Pittsburgh. Small gauge needles were placed in veins of each forearm for infusion and sampling of blood; each was kept patent with 0.9% saline. Oxytocin (Pitocin®, Parke Davis, Detroit, MI) was dissolved in 0.9% saline and administered via a Harvard infusion pump. Each patient received an 8 h infusion of OT and an 8 h infusion of saline (placebo) commencing at 09:00 h on 2 consecutive days. Infusions were administered using a double-blinded and randomized schedule. The initial rate of the OT infusion was 1 mU/min and the rate was increased by 1 mU/min every 40 min to a final rate of 12 mU/min. The placebo infusion of saline was administered in the same manner. During OT and placebo infusions, blood was sampled every 40 min for measurement of OT. Blood was sampled for measurement of LH every 20 min in the 5 women studied in the follicular phase of the menstrual cycle and every 10 min in the 5 men and the 5 women studied in the luteal phase of the cycle. None of the men and women in the study experienced adverse effects during the study.

The study was approved by the Review Board for Biomedical Research at the University of Pittsburgh. Informed written consent was obtained from each individual.

**Radioimmunoassay**

Plasma OT was measured by RIA after acetone/ether extraction of plasma using methods previously published (17,18). The antiserum to OT used in this study was OT Ab-2 which was generated in the Pittsburgh laboratory by injection of synthetic OT/egg albumin mixture into rabbits. The antiserum to OT is specific for OT (18). The antiserum to OT was diluted to final dilution of 1:100 000 in 0.01 mol/l potassium phosphate buffer containing 8.7 g NaCl and 0.25% BSA, pH 7.4. Binding of labelled OT in the absence of unlabelled hormone averaged 40%, and nonspecific binding was always less than 4%. The intra- and inter-assay coefficients of variation less than 5 and 10%, respectively. The standard used in the OT assay was USP pituitary reference standard (US Pharmaceuticals, Rockville, MD).

The dried extracts of plasma were dissolved in 500 μl KPO4 buffer. Duplicates of 100 μl of each plasma extract were placed in glass tubes, 100 μl antiserum was added, and potassium phosphate buffer was added to make a total volume of 500 μl. After incubation for 24 h at 4°C, 50 μl radiolabelled OT was added to each tube, and the incubation was continued for another 4 days at 4°C. Bound and free hormone were separated by precipitation with 25% polyethylene glycol containing rabbit gammaglobulin (20 g/l). Precipitates were counted for 5 min in a gamma-counter. The radioactivity in the RIA tubes was corrected for nonspecific binding.

LH was measured in serum by RIA using methods previously described (19,20). The standard used in the assay was the pituitary gonadotropin standard LER-907. The intra- and inter-assay coefficients of variation were less than 8 and 12%, respectively. Samples for comparison from each patient were measured in one RIA.

![Graph](image.png)

**Fig. 1.**

LH secretion in 5 men during saline and oxytocin infusions. LH (lower panels of the graph) is expressed as percent of the mean LH during each 8 h infusion. Saline and oxytocin infusions are indicated at the top of the graph. The stepwise increase in the rate of the oxytocin infusion (mU/min) is indicated in the top right panel of the graph. The plasma oxytocin concentrations remained at basal levels during the saline infusion and increased during the oxytocin infusion as shown by the dashed lines in the upper panels of the graph. LH secretion in the 5 men was similar during saline and oxytocin infusions.
Statistical analysis and analysis of LH pulsatile secretion
LH pulsatile secretion was analyzed using the Detect program, version 4.6 (21). This program calculates the first derivative to detect sharp upstrokes (instantaneous secretory rate) and uses a curvefitting program to detect the exponential decay after a peak. We used an automatic determined baseline, tolerance and peak detection mode at a false positive rate of 5%. The median sn (5–7%) squared of the replicates of each was used for the AO term.

In agreement with previous results (22), LH pulse amplitudes were markedly heterogenous in women studied in the luteal phase, and ranged from 5 to 55 μg/l, with a median of 10 μg/l. Values of greater than 20 μg/l constituted 12% of scored pulses, and were arbitrarily designated 'large' LH pulses. The effects of oxytocin infusion on LH secretion in women in the luteal phase were also determined independently for small and large LH pulses.

Data are expressed as mean ± sd. Statistical significance was determined by Student's paired t-test. Results were considered significant if P = 0.05 or less.

Results
Plasma OT concentrations during saline infusion ranged from 1–3 fmol/l. Fig. 1 gives the transverse mean values for OT and LH among the normal men. No change in OT levels was found during the 8-h saline infusion, whereas OT levels rose linearly from 2–70 fmol/l during infusion of synthetic OT. Similar results were obtained for women in the follicular and luteal phase of the cycle.

The influence of synthetic OT vs saline infusion on mean LH concentrations, LH pulse frequency and amplitude is summarized in Table 1. We found no significant difference in mean LH levels, the number of LH pulses occurring in the 8-h sampling, nor in the mass of LH secreted per pulse as expressed as the area under the LH curve, in any of the three patient groups on the day of saline infusion compared to OT infusion day. Further, the linear increase in mean OT levels produced by the OT infusion was without effect on the transverse pattern of LH secretion in men (Fig. 1) or in women (not shown). The order of the infusion, OT before saline or OT after saline, also had no significant effect (P > 0.05) on the LH parameters examined.

When LH pulses in women in the luteal phase were divided into pulses of small and large amplitude, neither was influenced by OT. The frequency of small pulses in women in the luteal phase was similar to the LH pulse frequency in women in the follicular phase during both saline and OT infusion, whereas the frequency of large LH pulses, which was also unchanged by OT, was approximately 1/8 h. LH pulse frequency in the men studied was slightly, but not significantly (P > 0.05) greater than that of women in the follicular phase.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Infusion</th>
<th>Mean LH concentration (μg/l)*</th>
<th>Mean pulse frequency (pulses/8 h)</th>
<th>Mean pulse area (μg · l⁻¹ · min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men (N = 5)</td>
<td>Oxytocin</td>
<td>20.8 ± 4.6</td>
<td>10.2 ± 3.2</td>
<td>74 ± 9</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>21.4 ± 4.6</td>
<td>9.2 ± 3.0</td>
<td>78 ± 9</td>
</tr>
<tr>
<td>Women (N = 5)</td>
<td>Follicular</td>
<td>Oxytocin</td>
<td>70 ± 8.4</td>
<td>5.6 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>60 ± 7.7</td>
<td>5.8 ± 2.4</td>
<td>192 ± 14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large pulses</td>
<td>Small pulses</td>
<td>Large pulses</td>
<td>Small pulses</td>
<td></td>
</tr>
<tr>
<td>Luteal</td>
<td>Oxytocin</td>
<td>23.5 ± 4.8</td>
<td>1.2 ± 1.3</td>
<td>340 ± 70</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>24.4 ± 4.9</td>
<td>1.0 ± 1.2</td>
<td>7.4 ± 3.7</td>
</tr>
</tbody>
</table>

All values given are mean ± sd.

* Mean of 48 samples drawn every 10 min for 8 h in 5 men and 5 luteal phase women and 24 samples drawn every 20 min for 8 h in 5 follicular phase women.
Discussion

In the present study, we found no effect of peripheral OT administration on mean LH levels or on the pulsatile pattern of LH secretion in normal men or women studied during the follicular or luteal phases of the menstrual cycle. The use of an increasing rate of infusion of OT allowed for examination of LH secretion over a broad range of physiological plasma OT concentrations. Several investigators (13–16) previously found that peripheral administration of pharmacological doses of synthetic OT had no effect upon basal LH or GnRH-stimulated LH release in humans. Now the failure to find an effect of infused OT with a more comprehensive examination of LH secretion, coupled with previous findings, suggests that an increase in the peripheral concentration of OT does not affect the LH-producing cells of the anterior pituitary gland. Whether OT affects the GnRH pulse generator cannot be determined in humans because circulating OT does not cross the blood-brain barrier.

Our estimate of LH pulse frequency in men in this study is somewhat greater than that recently published for normal men using a similar sampling protocol in which the results were analyzed with the Detect algorithm (23). The small LH pulses scored using intensive sampling protocols may represent true signals or false positive fluctuations. In this study, insufficient serum was available for the cross correlation with α subunit pulses (24) a method of analysis which may distinguish between these possibilities.

The results in humans are in agreement with a study in the bovine species which also found no significant effect of OT infusion on mean LH, the number and amplitude of LH pulses, and the area under the LH curve (25). Both our study and that of Irvin et al. 1981 (25) are in contrast to in vitro studies in the rat in which OT stimulated LH release (9). Possible explanations for the differences include variation among species in the effects of OT upon LH neurons as well as differences in experimental design. In addition, because the concentration of OT in portal blood bathing the anterior pituitary may be considerably higher than that achieved in the present study, we cannot exclude a possible paracrine action of hypothalamic OT on pituitary LH neurons or GnRH neurons. We conclude that it is unlikely that increases in plasma OT at the concentrations achieved and for the period of observation in this study regulate pulsatile LH secretion in humans.

Acknowledgments

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References


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