Neurohypophysial secretion to insulin-induced hypoglycemia and its regulation by endogenous opioids in women

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Abstract. In animals, there is sexual dimorphism of both neurohypophysial peptide secretion in response to stressful stimuli and to the inhibitory effects of opioids. In men, endogenous opioids inhibit the release of oxytocin when AVP secretion is stimulated by insulin-induced hypoglycemia. We have now investigated the role of endogenous opioids in the AVP and oxytocin response to insulin-induced hypoglycemia in women. Twelve subjects, 6 in the follicular and 6 in the luteal phase of the menstrual cycle, were infused on 2 occasions with naloxone (4 mg bolus and 6 mg/h) or saline. Soluble insulin (Human Actrapid®, 0.15 μ/kg, iv) was given and serial blood samples taken. Blood sugar fell significantly (p<0.05) and similarly in all groups. In the follicular phase hypoglycemia led to a rise in plasma AVP from 1.3 ± 0.2 to 1.8 ± 0.2 pmol/l in the saline-infused subjects (NS), and from 1.0 ± 0.1 to 2.0 ± 0.2 pmol/l in the naloxone-infused (p<0.05). AVP rose similarly from 0.6 ± 0.1 to 1.6 ± 0.5 pmol/l (p<0.05) in the luteal phase controls and from 0.8 ± 0.1 to 1.5 ± 0.3 pmol/l (p<0.05) in naloxone-infused subjects in the luteal phase. There were no significant differences between any of these groups. There were no significant changes in plasma oxytocin in any group. We therefore conclude that in women, unlike men, endogenous opioids do not modulate oxytocin or vasopressin release during insulin-induced hypoglycemia.

Sex differences in neurohypophysial response to immobilisation stress have been reported in experimental animals. Female rats release both vasopressin (AVP) and oxytocin (OT) in response to immobilisation, while in male rats the release of OT is considerably smaller and there is no significant release of AVP (1). Naloxone potentiates the release of both OT and AVP in females, in intact males there is no potentiation. In castrated males, however, stress-induced OT release is enhanced by naloxone (2). In female goats OT response to vaginal distension is potentiated by estrogen, but reduced by progesterone administration (3,4), and is also potentiated by an opioid antagonist (5). The ascending adrenergic pathways also have sexually dimorphic roles in the neurohypophysial response to stress (6). Animal studies therefore suggest that the sexually dimorphic OT and AVP responses to a variety of stimuli are determined by interactions between sex hormones and the opioid and central catecholamine pathways.

There is also evidence for sex hormone and opioid regulation of neurohypophysial hormone release in man. In men OT but not AVP release was increased by an opioid antagonist during insulin-induced hypoglycemia (7). In women breast stimulation in the luteal but not the follicular phase of the menstrual cycle stimulates OT secretion (8); and in children, the response of AVP to hypoglycemia is absent before puberty (9). We have now used the same stimulus in women to investigate whether a sex difference exists in the neurohypophysial response to hypoglycemia, whether the response changes during the menstrual cycle, and whether it is modulated by endogenous opioid peptides.
Subjects and Methods

Twelve non-smoking women, 6 in the follicular phase and 6 in the luteal phase of the menstrual cycle, were investigated twice, 48 h apart. All gave informed consent, and none were taking either regular medication or oral contraceptives. Serum progesterone levels were measured to confirm that subjects were in the luteal phase of the cycle. Subjects were recumbent for 30 min prior to study, antecubital veins were cannulated for sampling and infusion, and blood pressure and pulse were recorded automatically throughout. A baseline blood sample was taken and infusion with either naloxone (4 mg bolus and 6 mg/h) or saline (in random order) commenced and continued until the end of the study. After 30 min another blood sample was taken and soluble insulin (Human Actrapid®, 0.15 μ/kg) given iv. Further blood samples were taken 20, 30, 45 and 60 min after insulin. The study was repeated using the remaining infusion.

Blood samples were taken into fluoride tubes for blood sugar estimation and into lithium heparin tubes on ice. The latter were spun immediately at 4°C, and the plasma separated and stored at −20°C until peptide estimation. Plasma was extracted through octadecaslyl silica cartridges (Sep-pak, Waters, MA); extraction yield was greater than 90% for both AVP and OT. Both peptides were estimated in sensitive and specific radioimmunoassays (10). Intra-assay coefficients of variation were <5% for AVP and OT (2 fmol standard, 10 replicates), interassay coefficient of variation was 10% for OT and 15% for AVP (2 fmol standard). Cross-reactivity with the other peptide was <0.1%. The lowest detectable level of AVP was 0.2 pmol/l and of OT 0.4 pmol/l.

Statistics

Data were assessed by analysis of variance and Duncan’s multiple range test. Significance was set at p<0.05. Values are means ± SEM.

Results

All subjects experienced symptomatic neuroglycopenia; some became drowsy and were given dextrose, none was nauseated. Blood pressure fell in all groups during the study, but this fall was only significant in the luteal phase subjects (Table 1), heart rate rose significantly and similarly in all groups (Table 2). The blood sugar fell significantly in all groups between time 20 and 30 min after insulin; there was no difference between the groups (Table 3). Naloxone infusion alone had no effect on unstimulated AVP or OT levels (Table 4). In the follicular phase there were similar small rises of AVP in saline- and naloxone-infused subjects, although

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Follicular</th>
<th>Luteal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>Naloxone</td>
<td>Saline</td>
</tr>
<tr>
<td>0</td>
<td>89±5</td>
<td>90±2</td>
</tr>
<tr>
<td>20</td>
<td>85±4</td>
<td>89±2</td>
</tr>
<tr>
<td>30</td>
<td>87±4</td>
<td>90±3</td>
</tr>
<tr>
<td>45</td>
<td>83±3</td>
<td>84±5</td>
</tr>
<tr>
<td>60</td>
<td>81±3</td>
<td>81±3</td>
</tr>
</tbody>
</table>

* Fall significant p<0.05 vs time 0 min.

Table 2.

Heart rate (beats/min) response to hypoglycemia during saline or naloxone infusion in the luteal and the follicular phases of the menstrual cycle.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Follicular</th>
<th>Luteal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>Naloxone</td>
<td>Saline</td>
</tr>
<tr>
<td>0</td>
<td>75±3</td>
<td>76±3</td>
</tr>
<tr>
<td>20</td>
<td>84±6</td>
<td>79±4</td>
</tr>
<tr>
<td>30</td>
<td>92±3*</td>
<td>98±5*</td>
</tr>
<tr>
<td>45</td>
<td>82±6</td>
<td>82±5</td>
</tr>
<tr>
<td>60</td>
<td>81±3</td>
<td>84±4</td>
</tr>
</tbody>
</table>

* Increase significant p<0.05 vs time 0 min.

Table 3.

Baseline and mean nadir of blood sugar (mmol/l) levels during saline and naloxone infusions in the follicular and luteal phases of the menstrual cycle.

<table>
<thead>
<tr>
<th></th>
<th>Follicular</th>
<th>Luteal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>Naloxone</td>
<td>Saline</td>
</tr>
<tr>
<td>Baseline</td>
<td>4.4±0.2</td>
<td>4.5±0.1</td>
</tr>
<tr>
<td>Nadir</td>
<td>1.2±0.2</td>
<td>1.0±0.05</td>
</tr>
</tbody>
</table>

Significant fall from baseline in all groups p<0.05.
Fig. 1.
The changes in plasma AVP in response to insulin-induced hypoglycemia in the follicular (upper) and the luteal (lower) phases of the menstrual cycle in subjects infused with either saline – – or naloxone (4 mg bolus and 6 mg/h) – –. Points are means ± SEM.

Fig. 2.
The changes in plasma oxytocin (OT) in response to insulin-induced hypoglycemia in the follicular (upper) and the luteal (lower) phases of the menstrual cycle in subjects infused with either saline – – or naloxone (4 mg bolus and 6 mg/h) – –. Points are means ± SEM.

Discussion
When AVP secretion is stimulated by insulin-induced hypoglycemia in men OT release is inhibited by endogenous opioid peptides (7). We now report that in women insulin-induced hypoglycemia stimulates AVP release but not OT in both phases of the menstrual cycle, and that neither OT nor AVP release to this stimulus is inhibited by endogenous opioids.

Female rats respond to immobilisation stress with a marked release of OT and a smaller release of AVP, while males respond with a much smaller release of OT only. Ovariectomy has no effect on this response, but castration increases the OT response of males (1). Neonatal castration does not alter the OT response in males, but a single injection of testosterone to female neonates results in a male
pattern of response (11). Testosterone therefore inhibits OT secretion in response to stress both by an organisational effect in the neonate (possibly on ascending nor-adrenergic pathways (6)), and by a direct effect in the adult. The ability of naloxone to enhance OT release in females and castrated males (2) suggests an interaction between sex hormones and opioid-mediated inhibition of OT release in this species. In the goat, OT secretion in response to vaginal distension is also increased by estrogen infusion and reduced by progesterone infusion (3,4). Therefore both progesterone and estrogen may also differentially influence OT release to a stimulus which is also opiate-dependent in this species (5).

Insulin-induced hypoglycemia has been advocated as a test of AVP release in man (12). Some studies have failed to show OT release to hypoglycemia in man (7,13), although OT secretion in response to hypoglycemia alone has been reported in normal men (14), and in male and female insulin-dependent diabetic patients (15). In the present study we have not seen OT release to this stimulus. Perhaps the difference between the studies reflect the varying degrees or duration of neuroglycopenia induced (13). In the present study, hypoglycemia was more marked in both our current female subjects than in our previously reported findings in men (7).

Women taking estrogen in the form of the oral contraceptive pill have higher basal OT values, and acute ingestion of estrogen results in an increase in OT levels (16). Exogenous estrogen would therefore seem to enhance basal OT release, although the evidence that the variation in endogenous estrogens during the menstrual cycle effects OT release is conflicting (17,18). In our previous studies in males we have used nicotine (19) and insulin induced-hypoglycemia (7) and demonstrated that naloxone increases OT release when AVP is released in response to these acute stimuli. In women using breast stimulation and breast feeding to stimulate OT secretion we found no effect of naloxone (20). The results of the present study demonstrate that in women in either the follicular or the luteal phase of the menstrual cycle endogenous opioids do not inhibit OT or AVP release during insulin-induced hypoglycemia and there was no significant response of AVP to hypoglycemia during the follicular phase. There does therefore appear to be a sexually dimorphic response of neurohypophysial hormones to hypoglycemia, which in females is dependent on the phase of the menstrual cycle. Endogenous opioid peptides also only seem to be active in the inhibition of hypoglycemia-induced OT secretion in the male.

References


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