Effect of opioid peptides on gonadotrophin secretion

M. Motta and L. Martini

Department of Endocrinology, University of Milano, 21, Via A. del Sarto, 20129 — Milano, Italy

Abstract. The intraventricular injection of 25 µg of Methionine-Enkephalin (Met-Enk) induces a significant increase of serum LH levels in long-term ovariectomized rats 15, 30 and 60 min following administration. The synthetic Met-Enk agonistic analogue [D-Ala²]Methionine-Enkephalinamide ([D-Ala²]Met-Enk) also enhances significantly serum LH levels at 30 and 60 min; under the same experimental conditions neither Met-Enk nor [D-Ala²]Met-Enk modifies serum levels of FSH following intraventricular injections into ovariectomized animals. It is concluded that, under particular circumstances, opioid peptides of the Met-Enk family may stimulate LH release.

The presence of the opioid peptides (β-endorphin, Met-Enkephalin, etc.) in hypothalamic neurones (Wamsley et al. 1980; see Uhl et al. 1978 for additional references) has generated the idea that they might participate in the control of anterior pituitary function. There is now a consensus on the fact that they exert a stimulatory effect on growth hormone and prolactin secretion (see Meites et al. 1979 for references). The situation is less clear with regard to their participation in the control of gonadotrophin secretion. Barracough & Sawyer (1955) reported that morphine blocks ovulation in female rats if injected during a proper time of the day of pro-oestrus; this effect is reversed by naloxone, a potent opioid antagonist (Packman & Rothchild 1976). These data have been confirmed by Pang et al. (1977) and by Sylvester et al. (1980), who have shown, in addition, that morphine also prevents the physiological LH and FSH peaks, typical of the afternoon of pro-oestrus, as well as the LH surges induced, in the ovariectomized rats, by ovarian steroids. Morphine has also been reported to decrease serum LH levels in ovariectomized rats (Blank et al. 1980). On the basis of these data and of other indirect evidence derived from the utilization of the opioid antagonist naloxone it has been postulated that opioid peptides exert an inhibitory effect on LH secretion (Bruni et al. 1977; Cicero et al. 1980; see Meites et al. 1979 for additional references). However, several inconsistencies have appeared. For instance, it has been shown that, in pro-oestrous animals, the effects of morphine are dose-dependent, low doses enhancing and higher doses suppressing the spontaneous LH surges (Pang et al. 1977). Moreover, morphine has been found unable to modify serum LH levels in normal prepubertal or adult male rats (Cicero et al. 1979; Ieiri et al. 1979), as well as in prepuberal females (Ieiri et al. 1979). Also the effects of the opioid antagonists have been found to be highly variable, depending on the sex and the endocrine situation of the experimental animals (Bruni et al. 1977; Van Vugt et al. 1978; Ieiri et al. 1979; Blank et al. 1980; Grandison et al. 1980). Further contradictions emerge from the few studies in which the effects of the direct administration of the opioid peptides have been evaluated. Met-Enkephalin (Met-Enk) has been found to decrease circulating LH levels in the male rat (Bruni et al. 1977), while another opioid, β-endorphin, has been reported to have the opposite effect (Takahara et al. 1978).

The present investigation has been performed in order to gain additional information on the role of the opioid peptides in the control of the secretion of pituitary gonadotrophins. The approach selected has been that of injecting Met-Enk (the predominant opiate-like peptide in the rat) (Hong et al. 1977; Yang et al. 1977) and one of its
anallogues ([D-Ala²]Methionine-Enkephalinamide, [D-Ala²]Met-Enk) directly into the cerebrospinal fluid of castrated female rats and of measuring serum LH and FSH levels at various intervals following the administration of the compounds.

Material and Methods

Female rats of the Sprague-Dawley strain were housed in a temperature (22°C) and light-controlled room (10 h dark and 14 h light starting at 06.00). Food and water were available ad libitum. Animals were ovariectomized when they reached the weight of 170–180 g. Three weeks later they were implanted, in a lateral ventricle of the brain, with a cannula which was cemented to the skull to allow the administration of the opioid peptides or of the vehicle into the cerebrospinal fluid. The animals were starved the night preceding the experiment and were anaesthetized with pentobarbital (3 mg 100/g bw, ip) at about 09.00. Different groups of animals were then injected, through the cerebral cannula, with 25 μg of Met-Enk (Bachem, USA) or with 25 μg of [D-Ala²]Met-Enk (Bachem, USA) or with physiological saline. The two opioids were dissolved in saline and injected into the lateral ventricle in a volume of 10 μl. Animals were decapitated 15, 30 and 60 min later.

Blood was collected from the trunk vessels. Serum samples were kept frozen at –20°C, until the radioimmunoassays were performed. LH and FSH were measured respectively according to the procedures of Niswender et al. (1968) and of Daane & Parlow (1971). The data were statistically analyzed, utilizing the Dunnett test for multiple comparisons after one way analysis of variance (Dunnett 1955).

Results

It is apparent from Table 1 that the injection of 25 μg of Met-Enk into the cerebrospinal fluid of the lateral ventricles induced in long-term ovariectomized rats a significant increase of serum LH levels 15, 30 and 60 min following administration. A tendency towards a decrease of serum FSH levels was also observed, but such a decrease did not reach significance at any time interval considered. It is apparent from Table 1 that also [D-Ala²]Met-Enk enhanced serum LH levels when given in the

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time after injection</th>
<th>LH (NIH-LH S-17) ng/ml</th>
<th>FSH (NIAMDD-Rat FSH-RP-1) ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>15'</td>
<td>6.60 ± 0.33 (6)</td>
<td>1618 ± 94 (6)</td>
</tr>
<tr>
<td>Met-Enk</td>
<td>15'</td>
<td>11.85 ± 1.30* (6)</td>
<td>1498 ± 130 (6)</td>
</tr>
<tr>
<td>Saline</td>
<td>30'</td>
<td>7.11 ± 0.25 (6)</td>
<td>1530 ± 107 (6)</td>
</tr>
<tr>
<td>Met-Enk</td>
<td>30'</td>
<td>14.36 ± 1.38* (5)</td>
<td>1416 ± 103 (6)</td>
</tr>
<tr>
<td>Saline</td>
<td>60'</td>
<td>7.31 ± 0.18 (6)</td>
<td>1574 ± 133 (6)</td>
</tr>
<tr>
<td>Met-Enk</td>
<td>60'</td>
<td>12.67 ± 1.26* (5)</td>
<td>1365 ± 54 (5)</td>
</tr>
<tr>
<td>Saline</td>
<td>15'</td>
<td>10.15 ± 0.58 (6)</td>
<td>1427 ± 125 (5)</td>
</tr>
<tr>
<td>[D-Ala²]Met-Enk</td>
<td>15'</td>
<td>9.26 ± 1.80 (6)</td>
<td>1672 ± 106 (6)</td>
</tr>
<tr>
<td>Saline</td>
<td>30'</td>
<td>11.18 ± 0.34 (6)</td>
<td>1343 ± 148 (6)</td>
</tr>
<tr>
<td>[D-Ala²]Met-Enk</td>
<td>30'</td>
<td>19.02 ± 2.34* (5)</td>
<td>1124 ± 65 (6)</td>
</tr>
<tr>
<td>Saline</td>
<td>60'</td>
<td>11.54 ± 0.49 (6)</td>
<td>1482 ± 75 (6)</td>
</tr>
<tr>
<td>[D-Ala²]Met-Enk</td>
<td>60'</td>
<td>17.18 ± 1.98** (5)</td>
<td>1352 ± 86 (6)</td>
</tr>
</tbody>
</table>

Number of animals in parentheses. Values are means ± se. * P < 0.01 vs saline injected animals. ** P < 0.05 vs saline injected animals.
same experimental conditions; following the intraventricular administration of [D-Ala²]Met-Enk serum LH levels were significantly higher than in the control groups beginning at 30 min after administration. As in the case of Met-Enk injections there was no significant effect on FSH secretion.

Discussion

The present data show that Met-Enk and one of its analogues may increase LH secretion in female rats, when the compounds are directly injected into the cerebrospinal fluid. The fact that this stimulatory effect occurs in castrated animals, whose serum LH levels are already elevated, makes the results particularly significant. The effect of the two opioids on LH release does not seem to be accompanied by any significant effect on FSH secretion.

The present data are in agreement with those reported in the only other study in which the intraventricular way of administration of the opioids has been used. Takahara et al. (1978) found the intraventricular injection of a small dose of β-endorphin (5 µg) to cause a significant increase in serum LH levels in adult male rats sampled 30 min after the injection. At first glance, the present results appear to be at variance with those of Bruni et al. (1977), who reported that Met-Enk depresses LH secretion. However, the discrepancy between the two groups of results may easily be explained by major methodological differences. First of all, Bruni et al. (1977) used normal adult male rats rather than castrated females. Secondly, they used a dose of the peptide (5 mg) which may be regarded as pharmacological (Lee et al. 1980), when compared to the one (25 µg) utilized here. Thirdly (and this is probably the major and more important difference) Bruni et al. (1977) injected the compound peripherally rather than intracerebrally. It is obvious that a compound given systemically (especially if in a high dose) may have a distribution in the brain different from that of the same compound given intraventricularly in minute amounts.

The brain distribution of the injected peptides becomes of relevance if one postulates that, in the brain, there are different opioid systems, which exert opposite effects on the LRH-producing neurones (inhibition or activation). Following systemic administrations of huge doses, both systems might be affected and the possible prevalence of the inhibiting one might appear. On the contrary, the local injection of minute amounts might bring compound to act only on one system (in our case the stimulatory one). In this context, it is of relevance to recall that both excitation and inhibition have been found in rat hypothalamic neurones following acute morphine administration (Kerr 1974), and that Pang et al. (1977) have already suggested that these different units might participate in opposite directions in the neurogenic control of anterior pituitary functions. It is also interesting to underline that in Bruni’s experiments naloxone could only partially counteract the inhibitory action of Met-Enk on LH release (Bruni et al. 1977). This observation opens the possibility that different types of opioid receptors (endowed respectively with inhibiting and with stimulating properties) might be devoted to the control of LH secretion. Evidence for the existence of multiple opioid receptors is rapidly accumulating (Pasternak et al. 1980).

Our contention that differences in the doses of the opioids might play a role in directing their effect on LH secretion is certainly supported by the findings of Pang et al. (1977), who noted that the systemic administration of low doses of morphine (10 mg/kg) facilitates the release of LH in the pro-oestrous rat while much higher doses (60 mg/kg) will suppress the secretion of this hormone.

The stimulatory role of Met-Enk on LH release reported here might have a physiological significance, and participate in evoking the pro-oestrous LH surge. Kumar et al. (1979) have found that Met-Enk stores decrease in the anterior hypothalamic-preoptic area and in the mediobasal hypothalamus during the day of pro-oestrus prior to the LH surge. If such a decrease reflects the release of the opioids at neuronal junctions, an activation of LRH secretory neurones would appear justified.

A stimulatory effect of opioid peptides on LRH secretion might have been predicted on the basis of their mechanism of action. Recent evidence indicates that the opioids interfere with dopaminergic and cholinergic systems in the brain. The opioids decrease both the storage and the turnover of dopamine in the median eminence (see Van Loon et al. 1980 for references), and consequently may eliminate the inhibitory tone dopamine exerts on LRH release (see Drouva & Gallo 1977 for references).

Moreover, antimuscarinic drugs which cross the blood brain barrier have been reported to block the
stimulatory effect enkephalinergic agonists exert on growth hormone secretion (Casanueva et al. 1980), a result which indicates that a cholinergic step is involved in the stimulatory effects the opioids exert on the hypothalamic-pituitary axis. It is known since several years that acetylcholine is a major stimulus for the release of LRH (Simonovic et al. 1974; Fiorindo & Martini 1975; Justo et al. 1975).

The present experiments show that opioids of the enkephalin family seem to affect LH release without modifying FSH secretion. A differential effect of morphine, Met-Enk and their antagonists on LH and FSH secretion has been observed also by those investigators who claim that the opioids inhibit LH secretion (Bruni et al. 1977; Pang et al. 1977). This fact is somewhat puzzling, particularly if only one hypothalamic releasing factor exists for the control of the secretion of the two gonadotrophins.

It remains for further work to establish how the opioids may interfere with the secretion of only one gonadotrophin, especially in the light of the well documented fact that these principles do not interfere with the effects of LRH at pituitary level (Cicero et al. 1977; Pang et al. 1977).

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