Effects of naloxone and neonatal treatment with monosodium-L-glutamate on growth hormone and prolactin release induced by electrical stimulation of the medial-basal hypothalamus in rats

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(Received 16 June 1982)

SUMMARY
The role of nerve cells of the arcuate nucleus and endogenous opioid peptides in the regulation of GH and prolactin secretion has been investigated. Electrical stimulation of the medial-basal hypothalamus (MBH) for 10 min raised plasma levels of both hormones in male rats anaesthetized with pentobarbitone sodium. Plasma hormone levels increased within 5 min after the termination of the stimulus, while no marked changes were found during stimulation. The GH response to the electrical stimulus was substantially reduced in rats with arcuate lesions induced by neonatal treatment with monosodium-L-glutamate (MSG). By contrast, the size of the prolactin response was not altered by MSG treatment. The opiate receptor antagonist naloxone (10 mg/kg, i.v.) failed to influence GH secretion induced by electrical stimulation in either control or MSG-treated animals. The post-stimulus rise of plasma prolactin levels was attenuated by naloxone in control rats, while the same dose of the drug was ineffective in rats which had been exposed to MSG. We conclude that endogenous opioids participate in the increase of prolactin release upon electrical stimulation of the MBH but are not involved in the GH secretory response. Arcuate neurones are important in the maintenance of the GH response to electrical stimulation. By contrast, lesioning of the arcuate nucleus failed to affect the prolactin secretory response elicited by MBH stimulation. However, prolactin release in MSG-treated rats appeared less susceptible to the inhibitory action of naloxone, suggesting a possible supersensitivity towards endogenous opioids.

INTRODUCTION
The secretion of growth hormone (GH) and prolactin is stimulated by compounds having opiate activity (cf. Dupont, Cusan, Ferland, Lemay & Labrie, 1979). It appears that the principal site of action of morphine and methionine-enkephalin in stimulating GH and prolactin release is within the medial-basal hypothalamus (MBH) (Guidotti & Grandison, 1980; Belchetz, 1981; Haskins, Gudelsky, Moss & Porter, 1981). Nerve cells containing and most probably producing endogenous opioid peptides such as β-endorphin and enkephalins have been found in the MBH, particularly in the arcuate nucleus (Bloom, Battenberg, Rossier, Ling & Guillemin, 1978; Liotta, Gildersleeve, Brownstein & Krieger, 1979; Wamsley, Young & Kuhar, 1980). Endogenous opioids are also present in nerve terminals within the MBH, and β-endorphin is released by incubated hypothalami as well as by synaptosomal fractions of the hypothalamus (Fukata, Nakai & Imura, 1980; Vermees, Mulder, Berkenbosch & Tilders, 1981). It therefore seems quite plausible that endogenous opioids produced and released within the MBH may participate in the hypothalamic regulation of hormone secretion by the anterior pituitary gland. However, in-vivo evidence
suggesting the release of endogenous opioids within the MBH and their possible relationship to the secretion of GH and prolactin is scarce. In the present study we have investigated whether endogenous opioids are involved in the secretion of GH and prolactin induced by electrical stimulation of the MBH. We also have attempted to assess the role of the arcuate nucleus in electrically evoked hormone release by using rats neonatally treated with the neurotoxic excitant monosodium-L-glutamate (MSG) (Olney, 1969).

**MATERIALS AND METHODS**

**Animals**

Newborn CFY male albino rats bred in our Institute were injected with an 80% (w/v) solution of MSG (Renanal, Budapest, Hungary; 4 g/kg body weight, s.c.) or with 50% (w/v) NaCl (0·5 g/kg) on days 2, 4, 6 and 10 after birth. The animals were weaned at 21 days and kept in an air-conditioned room until use at 8–9 weeks. Pelleted rat chow and tap water were available *ad libitum*.

**Electrical stimulation**

Anaesthesia was induced with pentobarbitone sodium (Nembutal; Serva, Heidelberg, Germany; 35–40 mg/kg, i.p.) and a femoral vein was cannulated with a piece of Silastic tubing (Dow Corning, Midland, Michigan, U.S.A.). The animal was placed in a stereotaxic frame and a side-by-side bipolar electrode (interpolar distance 1·4 mm) was advanced into the MBH as previously described (Antoni, Makara, & Rappay, 1981). Electrical stimulation was started 20 min after the placement of the electrode. Stimulation lasted 10 min and consisted of 5-s trains of biphasic constant-current pulses (amplitude ± 200 µA, width 2 ms, frequency 60 Hz) alternating with 10-s pauses. Blood samples were drawn immediately before the onset of stimulation and 5, 10, 15 and 20 min afterwards. At the end of the experiment the brain was removed from the skull and processed for histology to verify the position of the electrodes, according to criteria previously described (Antoni, Makara & Rappay, 1981). Naloxone HCl (gift of Endo Laboratories, Garden City, New York, U.S.A.) was dissolved in 0·15 mol NaCl/l (saline) on the morning of the experiment and injected intravenously 3 min before the onset of stimulation; matched control animals received saline (2 ml/kg) intravenously. Saline (0·5 ml) was injected as volume replacement after each blood sampling.

**Hormone assays**

Plasma GH and prolactin were determined by radioimmunoassays using NIAMDD materials, the reference preparations being GH-RP-1 and PRL-RP-1 respectively. In the case of GH, separation of bound and free hormone was achieved by a heat- and formaldehyde-inactivated suspension (20%, w/v) of *Staphylococcus aureus* (Bact-A-Sorb; Humán, Budapest, Hungary) while for prolactin a second antibody raised in sheep against rabbit immunoglobulin G was used. The limits of detection for GH and prolactin were 6–12 and 20 µg/l plasma respectively.

**Statistics**

Plasma hormone data were evaluated after logarithmic transformation. Two-way analysis of variance followed by Dunnett’s test for multiple comparisons were performed. The data are presented as geometrical mean ± r.s.e.m., where r.s.e.m. indicates the retransformed standard error of the means of the logarithms of plasma hormone values.
RESULTS

The brains of MSG-treated rats showed the characteristic macroscopic (atrophy of the optic nerve, dilatation of the floor of the third cerebral ventricle) and microscopic (loss of nerve cell bodies in the arcuate nucleus) signs of the treatment.

In both groups of animals (MSG- and NaCl-treated) plasma GH levels rose abruptly within 5 min after the completion of the electrical stimulus, but did not change markedly during stimulation (Table 1). The prestimulation as well as the peak levels of GH (15 min) were much lower in rats which had been exposed to MSG. Naloxone (2 or 10 mg/kg) had no effect on the electrically induced rise of plasma GH levels in either group of rats and the

Table 1. Plasma GH and prolactin levels in rats neonatally treated with hypertonic NaCl or monosodium-l-glutamate (MSG) upon electrical stimulation of the medial-basal hypothalamus. Stimulation was carried out between 0 and 10 min, naloxone having been given intravenously 3 min before stimulation. Values are geometrical means ± R.S.E.M. (see Methods). Numbers of rats per group are shown in parentheses

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Naloxone (mg/kg)</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
</tr>
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<tbody>
<tr>
<td>Plasma GH (μg/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>NaCl</td>
<td>0</td>
<td>32.5±1.70</td>
<td>17.3±1.70</td>
<td>16.5±1.62</td>
<td>511.3±1.33**</td>
<td>635.7±1.40** (7)</td>
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<td>16.7±1.86</td>
<td>9.8±1.74</td>
<td>7.9±1.63</td>
<td>561.7±1.85**</td>
<td>842.8±1.16** (4)</td>
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<td></td>
<td>10</td>
<td>22.9±1.31</td>
<td>ND</td>
<td>ND</td>
<td>690.9±1.29**</td>
<td>802.9±1.28** (6)</td>
</tr>
<tr>
<td>MSG</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>68.8±1.41</td>
<td>78.2±1.95 (10)</td>
<td></td>
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<tr>
<td></td>
<td>10</td>
<td>10.4±1.25</td>
<td>ND</td>
<td>ND</td>
<td>78.0±1.22**</td>
<td>62.3±1.31** (9)</td>
</tr>
<tr>
<td>Plasma prolactin (μg/l)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>NaCl</td>
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<td>42.2±1.08</td>
<td>41.7±1.09</td>
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<td>107.2±1.15**</td>
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<tr>
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<td>43.8±1.09</td>
<td>46.4±1.08</td>
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<td>40.3±1.09</td>
<td>39.0±1.13</td>
<td>68.0±1.17†</td>
<td>54.4±1.13† (6)</td>
</tr>
<tr>
<td>MSG</td>
<td>0</td>
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<td>36.6±1.12</td>
<td>36.5±1.11</td>
<td>92.5±1.27**</td>
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<td>34.8±1.14</td>
<td>35.5±1.12</td>
<td>81.4±1.08**</td>
<td>68.1±1.12** (10)</td>
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</table>

*P<0.05, **P<0.01 compared with the respective value at 0 min (two-way analysis of variance and Dunnett's test); †P<0.05 compared with the respective time-point in the NaCl group given no naloxone (Student's t-test). ND. Mean not determined because of the high proportion of data points below the sensitivity of the assay.

temporal pattern of hormone secretion was also unchanged (Table 1). Plasma prolactin levels changed with a time-course identical to that of GH (Table 1). Levels of prolactin in MSG-treated rats were not different from those of their litter-mate controls at any of the time-points examined (Table 1). Naloxone (10 mg/kg) attenuated the rise of plasma prolactin in the group treated with hypertonic saline while it had no effect in MSG-treated rats (Table 1).

DISCUSSION

The characteristic post-stimulus rise of plasma GH level upon electrical stimulation of the MBH is well known (Frohman, Bernardis & Kant, 1968; Martin, 1972; Antoni, Makara & Rappay, 1981). The probable mechanism of this temporal pattern is the simultaneous activation of both somatostatin- and somatoliberin-containing nerve terminals in the median eminence, resulting in an abrupt increase of GH secretion once the electrically driven release of somatostatin decreases at the end of the stimulation period (Antoni, Makara & Rappay, 1981).

Since the neurotoxical lesioning of the arcuate nucleus produces a decrease of somatoliberin bioactivity in the pituitary stalk median eminence (Acs, Antoni & Makara, 1982), it
was anticipated that MSG treatment would decrease the rise of plasma GH after electrical stimulation of the MBH. Accordingly, this is what we found in the present study, supporting the suggestion that the size of the GH response to the electrical stimulus is determined by the amount of somatoliberin released into the pituitary portal circulation (Antoni, Makara & Rappay, 1981). A marked pituitary deficiency in the capacity to secrete GH seems unlikely in rats treated with MSG; incubated quarters of the anterior pituitary glands of MSG-treated rats secreted normal quantities of GH and the stimulation of GH release by prostaglandin E₂ was also unaltered (F. A. Antoni, unpublished data).

Naloxone failed to influence plasma levels of GH either in control or in MSG-treated rats at any of the time-points studied. Since the doses of the antagonist used were rather high, it seems reasonable to conclude that endogenous opioids do not play a role in the release of GH upon electrical stimulation of the MBH.

By contrast, naloxone (10 mg/kg) reduced the size of the increase of plasma prolactin levels by about 50% in rats treated with NaCl. This suggests the involvement of endogenous opioids in the prolactin secretory response induced by electrical stimulation. Previously, we have found that the electrical stimulus releases prolactin releasing factor(s) (PRF) as well as dopamine from the median eminence and that the delay of the rise in prolactin levels can be attributed to dopamine (Antoni, Makara & Kanyicska, 1981; F. A. Antoni, G. B. Makara & B. Kanyicska, unpublished observations). Hence, naloxone could attenuate the rise of prolactin levels either by facilitating dopamine release or by inhibiting the secretion of PRF. It is well known that opiate agonists inhibit tuberoinfundibular dopamine release (Gudelsky & Porter, 1979; Van Loon, Ho & Kim, 1979), the site of action being within the MBH (Guidotti & Grandison, 1980; Belchetz, 1981; Haskins et al. 1981). However, the possibility that endogenous opioids promote the release of PRF and that this effect is also antagonized by naloxone cannot be ruled out. Indeed, some supportive, albeit indirect, evidence has been presented in this regard (Spampinato, Locatelli, Cocchi, Vicentini, Bajusz, Ferri & Müller, 1979; Koenig, Mayfield, Coppins, McCann & Krulich, 1980; Antoni, Makara & Kanyicska, 1981).

Neonatal treatment with MSG destroys up to 80% of the nerve cells in the arcuate nucleus (Holzwarth-McBride, Sladek & Knigge, 1976) and produces parallel decreases in the hypothalamic content of various neurotransmitters and neuropeptides (cf. Kizer, Nemeroff & Youngblood, 1978). As judged by biochemical and histological techniques, the tuberoinfundibular dopaminergic system is severely affected by neonatal MSG treatment (Holzwarth-McBride et al. 1976; Nemeroff, Konkol, Bisette, Youngblood, Martin, Brazeau, Rone, Prange, Reese & Kizer, 1977; Walaas & Fonnnum, 1978). The same has been reported for neurenes within the arcuate nucleus that contain opioid peptides (Krieger, Liotta, Nicholson & Kizer, 1979; Bodnar, Abrams, Zimmerman, Krieger, Nicholson & Kizer, 1980; Romagnano, Chafel, Pilcher & Joseph, 1982). Despite the marked structural damage, the tuberoinfundibular dopaminergic system appears to be functionally active in MSG-treated animals as shown by the ‘normal’ levels of plasma prolactin under basal conditions (Nemeroff et al. 1977; Antoni, Kanyicska, Mezey & Makara, 1982) and under pentobarbitone or urethane anaesthesia, which are known to influence dopamine release from the median eminence in an opposite manner (Pilotte, Gudelsky & Porter, 1980). Further, the prolactin response to electrical stimulation of the MBH which involves the action of endogenous opioids was also unaltered. These findings suggest that considerable adaptation or functional reorganization of hypothalamic regulatory mechanisms might take place in animals neonatally exposed to MSG. An indication for such an adaptation/reorganization could be the lack of the inhibition of electrically induced prolactin secretion by a dose of naloxone that was effective in control animals. This suggests a supersensitivity towards the prolactin-stimulating action of opioids. In agreement with this suggestion, we have found that the intravenous injection of an opiate (morphine hydrochloride) elicited prolactin secretion three times more effectively in
urethane-anaesthetized rats which had been exposed neonatally to MSG (Antoni et al. 1982).

We thank Dr A. F. Parlow and the NIAMDD for radioimmunoassay materials, Ms J. Helfferich and Ms I. Szabó for technical assistance, Dr Zs. Ács for supervision of GH assays and Mr G. Folly for help in statistical analysis.

REFERENCES


