Morphine Increases Hypothalamic Noradrenaline Turnover But Rather Decreases Its Enhancement Induced by Stress in Rats

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Summary: By measuring levels of noradrenaline (NA) and its major metabolite, 3-methoxy-4-hydroxyphenylethylene glycol sulfate (MHPG-SO₄), we investigated the effects of morphine on hypothalamic noradrenergic neurons in stressed or non-stressed states in male rats. Subcutaneous administration of morphine at 3 mg/kg and 6 mg/kg reduced NA level and elevated MHPG-SO₄ level in the hypothalamus in a dose-dependent manner and these effects of morphine were completely abolished by pretreatment with naloxone at 0.5 mg/kg and 5 mg/kg (s.c.). Immobilization stress caused significant reduction of NA and increase in MHPG-SO₄ level, and this stress-induced increase in NA turnover was attenuated by pretreatment with 3 mg/kg and 6 mg/kg of morphine. Attenuation of stress-induced enhancement of hypothalamic NA turnover by morphine was almost completely reversed by naloxone at 0.5 mg/kg and 5 mg/kg. These results suggest that morphine produces different effects on hypothalamic noradrenergic neurons, depending on the state of the animal treated, i.e., facilitation in the non-stressed state and inhibition in the stressed state and that these actions occur via opiate receptors. It is further suggested that the hypothalamic opioid peptide system might be partially involved in the stress process by attenuating increased NA turnover by stress in rats.

Key words: immobilization stress — morphine — noradrenaline turnover — naloxone — hypothalamus

Introduction

By simultaneously measuring levels of noradrenaline (NA) and its major metabolite, 3-methoxy-4-hydroxyphenylethylene glycol sulfate (MHPG-SO₄) (Schanberg et al. 1968a, 1968b) which is considered a useful in assessing NA turnover in rat brain (Meek and Neff, 1973; Kohno et al. 1981), we reported that immobilization stress increases NA turnover in specific brain areas in rats including the hypothalamus, amygdala, thalamus, hippocampus, cerebral cortex and pons plus medulla oblongata (Tanaka et al. 1982). Among these regions, the most rapid and marked increase in NA turnover is observed in the hypothalamus.

Further, we noted that increased NA turnover in the hypothalamus by stress is enhanced by pretreatment with an opiate antagonist, naloxone 5 mg/kg, and suggested that endogenous opioid peptides in the hypothalamus might be partially involved in the stress process by attenuating the increase in NA turnover induced by stress (Tanaka et al. 1981).

However, there are an increasing num-
ber of reports that naloxone may have pharmacological actions unrelated to opiate receptors blockade, as reviewed by Sawynok et al. (1979).

If endogenous opioids, released during stress, attenuated the stress-induced increase of NA turnover in the rat hypothalamus, as suggested in our previous report using naloxone (Tanaka et al. 1981), morphine, a potent opiate, might also be expected to attenuate the stress-induced increase in NA turnover in the region.

The present study was undertaken to confirm the hypothesis and if possible, to determine using the opiate antagonist naloxone, whether or not the morphine effect occurs via opiate receptors.

Methods

Male Wistar rats (170 - 200 g) were housed in a 12 hour (7 a.m. to 7 p.m.) light/dark cycled room at constant room temperature (24 ± 1°C). Food and water were supplied ad libitum.

Immobilization stress was employed as a stress procedure by enclosing rats in a flexible wire mesh (3 × 3 mm) as described in the previous reports (Tanaka et al. 1981, 1982).

Two studies were performed separately. In the first study, rats were allocated to one of the following six groups. Those in the first three groups were injected with saline or with morphine at 3 mg/kg or 6 mg/kg 70 min before sacrifice. The animals in the remaining three groups were exposed to 1-hour immobilization stress 10 min after injection with saline or with the respective doses of morphine.

In the second study, rats pretreated with naloxone 0.5 mg/kg or 5 mg/kg were injected with morphine 6 mg/kg and half of them were exposed to immobilization stress for 1 hour. Naloxone and morphine were injected 70 min and 65 min before sacrifice, respectively. Control animals were injected with saline at the same time when naloxone or morphine was injected and half of them were exposed to stress for the same period.

Naloxone hydrochloride (a gift from Sankyo K.K.) and morphine hydrochloride (Sankyo K.K.) were dissolved in physiological saline and then injected subcutaneously in a volume of 0.2 ml/100 g body weight. Dosages used refer to the free base.

The rats were sacrificed by decapitation immediately after each treatment, and the hypothalamus was dissected out according to the method of Gispen et al., (1972) and stored at -45°C until assayed. NA and MHPG-SO₄ levels in the region were determined by our fluorometric method (Kohno et al. 1979).

Student's t-test (two-tailed) was employed for statistical analysis.

Results

As shown in Table 1, morphine at 6 mg/kg significantly reduced hypothalamic NA level (3 mg/kg; P < 0.10) and both doses of morphine significantly elevated MHPG-SO₄ levels in the region as compared to saline-unstressed rats. These changes occurred in a dose-dependent manner. Immobilization stress caused significant reduction of NA and elevation of MHPG-SO₄ level. Both doses of morphine significantly attenuated not only NA reduction but also MHPG-SO₄ elevation induced by stress as compared to saline-stressed animals. No statistically significant difference was obtained between morphine-unstressed and morphine-stressed rats.

In the second study, as similarly observed in the first study, morphine 6 mg/kg by itself significantly reduced NA level and increased MHPG-SO₄ level in the hypothalamus. Both NA reduction and MHPG-SO₄ elevation induced by morphine
### TABLE 1

**Effect of morphine on levels of the hypothalamic noradrenaline (NA) and 3-methoxy-4-hydroxy-phenylethynleglycol sulfate (MHPG-SO₄) (ng/g) in stressed and non-stressed rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NA</th>
<th>MHPG-SO₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>1746.8 ± 89.37 (8)</td>
<td>260.0 ± 11.62 (8)</td>
</tr>
<tr>
<td>Saline + Stress</td>
<td>1090.4 ± 33.56 (8)***</td>
<td>463.6 ± 20.00 (8)***</td>
</tr>
<tr>
<td>Morphine 3 mg/kg</td>
<td>1534.2 ± 79.20 (8)***</td>
<td>345.3 ± 15.82 (8)***</td>
</tr>
<tr>
<td>Morphine 3 mg/kg + Stress</td>
<td>1326.6 ± 62.52 (8)***</td>
<td>383.1 ± 15.62 (8)***</td>
</tr>
<tr>
<td>Morphine 6 mg/kg</td>
<td>1406.3 ± 71.71 (8)***</td>
<td>401.6 ± 27.25 (8)***</td>
</tr>
<tr>
<td>Morphine 6 mg/kg + Stress</td>
<td>1294.2 ± 61.06 (8)***</td>
<td>395.5 ± 21.71 (8)***</td>
</tr>
</tbody>
</table>

Each value indicates the mean ± S.E.M. of rats in each group of which number is shown in parenthesis.

Statistical significance:
- a: vs saline controls
- b: vs saline + stress
- †: not significantly different compared to respective controls (morphine alone)

Statistical significance levels are:
- * P < 0.05, ** P < 0.01, *** P < 0.001

See text for detail.

### TABLE 2

**Effects of morphine on levels of hypothalamic NA and MHPG-SO₄ (ng/g) in stressed and non-stressed rats and reversal of effects by naloxone pretreatment**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NA</th>
<th>MHPG-SO₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>1795.4 ± 69.26 (8)</td>
<td>242.4 ± 11.27 (8)</td>
</tr>
<tr>
<td>Morphine 6 mg/kg</td>
<td>1593.4 ± 59.11 (7)**</td>
<td>378.7 ± 21.98 (7)***</td>
</tr>
<tr>
<td>Morphine 6 mg/kg + Naloxone 0.5 mg/kg</td>
<td>1704.5 ± 62.60 (7)</td>
<td>242.0 ± 11.89 (8)b***</td>
</tr>
<tr>
<td>Morphine 6 mg/kg + Naloxone 5 mg/kg</td>
<td>1779.3 ± 78.31 (7)</td>
<td>240.0 ± 9.47 (8)b***</td>
</tr>
<tr>
<td>Saline + Stress</td>
<td>1321.3 ± 49.74 (7)***</td>
<td>500.0 ± 17.19 (8)***</td>
</tr>
<tr>
<td>Morphine 6 mg/kg + Stress</td>
<td>1554.0 ± 95.00 (6)***</td>
<td>375.1 ± 27.74 (8)c***</td>
</tr>
<tr>
<td>Morphine 6 mg/kg + Naloxone 0.5 mg/kg + Stress</td>
<td>1333.3 ± 59.26 (7)</td>
<td>465.7 ± 26.67 (8)d **</td>
</tr>
<tr>
<td>Morphine 6 mg/kg + Naloxone 5 mg/kg + Stress</td>
<td>1389.0 ± 24.13 (7)</td>
<td>491.7 ± 20.67(8)d **</td>
</tr>
</tbody>
</table>

Each value indicates the mean ± S.E.M. of rats in each group of which number is indciated in parenthesis.

Statistical significance:
- a: vs saline control
- b: vs morphine 6 mg/kg
- c: vs saline + stress
- d: vs morphine 6 mg/kg + stress

Statistical significance levels are:
- * P < 0.05, ** P < 0.01, *** P < 0.001

See text for detail.
at 6 mg/kg were completely reversed by pretreatment with both doses of naloxone, 0.5 mg/kg and 5 mg/kg. Morphine 6 mg/kg significantly but partially reversed both NA reduction and MHPG-SO₄ elevation induced by stress. Both doses of naloxone almost completely abolished the attenuating effect of morphine on increased MHPG-SO₄ level induced by stress and tended to attenuate the drug effect on reduced NA level by stress (P<0.10).

Discussion

In the present study, morphine at 3 mg/kg and 6 mg/kg reduced NA level and increased MHPG-SO₄ level in the hypothalamus in a dose-dependent manner. The result is consistent with previous findings which showed that acute morphine administration in dogs and cats causes depletion of hypothalamic NA (Vogt, 1954) and that morphine increases MHPG-SO₄ levels in the whole brain (Roffman et al. 1975) and in the hypothalamus, brain stem and cerebellum (Roffman et al. 1977). However, they found significant alterations in NA or MHPG-SO₄ levels when much larger doses, such as 60 mg/kg or 25 mg/kg, of the drug were used compared to the present study. These higher doses have been generally employed in a majority of previous studies concerned with morphine effects on brain NA metabolism. Morphine at 81 mg/kg is toxic and lethal to approximately 33% of rats (Roffman et al. 1975). Even though 25 mg/kg of morphine increases hypothalamic MHPG-SO₄ level (Roffman et al. 1977), this dose is rather high and it remains a possibility that the drug effect may be secondary, that is a toxic effect. However, in the present study morphine, which was employed in very small doses, 3 mg/kg and 6 mg/kg, common for inducing analgesia in rats (Goldstein et al. 1976; Carmody et al. 1979; Bodnar et al. 1980), increased hypothalamic MHPG-SO₄ levels; moreover, these increases were accompanied with reductions of NA levels. The difference may result from our assay method employed in the present study, which is sensitive enough to simultaneously detect 2 ng of MHPG-SO₄ and 5 ng of NA (Kohno et al. 1981).

The finding that morphine at doses commonly used for inducing analgesia in rats reduces NA level and increases MHPG-SO₄ level in the hypothalamus suggests that the primary effect of morphine is to enhance NA release in the hypothalamus, not as a secondary, toxic effect. Reduced NA levels appear to result from utilization of NA exceeding the amine synthesis, which was reported to be increased by morphine in the study of Clouet et al. (1970) using [¹⁴C] tyrosine. This is consistent with the report by Johnson et al. (1974) which noted that the drug at a larger dose, 60 mg/kg, causes an increase in NA turnover in rat hypothalamus.

The morphine-induced increase in hypothalamic NA turnover was completely reversed by pretreatment with an opiate antagonist, naloxone, at both doses, 0.5 mg/kg and 5 mg/kg. This suggests that morphine increases NA turnover in the hypothalamus via opiate receptors. Recently, multiple forms of opiate receptors are proposed to be present in the brain (Llord et al. 1977; Audigier et al. 1977; Terenius, 1977). Among these receptors, the µ receptor might be involved in the morphine effect on NA turnover in the hypothalamus, since even small doses of naloxone, such as 0.5 mg/kg completely reverse the alteration of NA metabolism induced by morphine.

In contrast to these changes in NA turnover by morphine in non-stressed state, morphine has the opposite effect in hypothalamic noradrenergic neurons in the stress situation. Immobilization stress caused a significant reduction of NA and
an increase in MHPG-SO₄ in the hypothalamus and the result confirms our previous reports (Tanaka et al. 1981, 1982). The stress-induced enhancement of hypothalamic NA turnover was partially reversed by pretreatment with morphine at 3 mg/kg and 6 mg/kg; both stress-induced reduction of NA and elevation of MHPG-SO₄ were significantly attenuated by morphine at 3 mg/kg and 6 mg/kg. This suggests that morphine acts to attenuate increased NA turnover in the hypothalamus by stress. The finding further supports our previous suggestion that endogenous opioids in the hypothalamus may be partially involved in the stress process by attenuating the increase in NA turnover induced by stress.

Morphine-induced attenuation of stress-induced increase in hypothalamic MHPG-SO₄ level was also almost completely reversed by pretreatment with both doses of naloxone, 0.5 mg/kg and 5 mg/kg. However, attenuation of stress-induced NA reduction by morphine tended to be reversed by both doses of naloxone but was not statistically significant (P<0.10). This is probably due to the inhibitory effect of naloxone on catecholamine synthesis (García-Sevilla et al. 1978). Since morphine alone is reported to facilitate NA synthesis (Clouet et al. 1970), the change in NA contents when morphine and naloxone are administered simultaneously appears to be complicated. Despite these actions of the drugs, the findings suggest that morphine attenuates the stress-induced increase in hypothalamic NA turnover and that the effect also occurs via opiate receptors.

The present study demonstrated that the effect of morphine on hypothalamic noradrenergic neurons is quite different, depending on the situation of the rats. Morphine enhances hypothalamic NA turnover in the non-stressed state but attenuates increased NA turnover in the stressed state. This suggests that morphine oppositely affects the function mediated or modulated by the noradrenergic system in the hypothalamus, depending on the animal's situation.

Redmond et al. (1979) reported that a brain noradrenergic system might be involved in anxiety in the monkey. Morphine relieves the distress associated with pain but may sometimes produce an unpleasant dysphoria, mild anxiety or fear in pain-free individuals (Seiden and Dykstra, 1977). Taken together with our findings, the discrepancy of the morphine effect might well be explained by different actions of morphine on the noradrenergic system, depending on the situations, i.e.; facilitation of the noradrenergic system when non-stressed, which leads to unpleasant dysphoria, mild anxiety or fear, and inhibition when stressed, which leads to relief of distress and pain.

From the present results, it must be emphasized that the greatest care should be exercised when behavioral or neurochemical findings of morphine obtained in stressed animals are interpreted and compared to neurochemical findings of the drug obtained in non-stressed animals. In particular, one requires caution in the study of analgesia, since animals are often exposed to stressful situations.

The present study further supports the suggestion that hypothalamic opioid systems in rats are partially involved in the stress process by attenuating increased NA turnover by stress and that this attenuation may be closely related to the relief of fear or anxiety.

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