Enhancement of Stress-Induced Increase in Hypothalamic Noradrenaline Turnover by Pretreatment with Naloxone in Rats

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Summary: The relationship between the increase in noradrenaline (NA) turnover and endogenous opioid peptides in the hypothalamus under stressful situations was investigated in rats using a specific antagonist of μ -opioids, naloxone. Male Wistar rats were injected subcutaneously with saline, naloxone at 1 mg/kg or 5 mg/kg 10 min before exposure to immobilization stress for 1 hour. Levels of NA and its major metabolite, 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-SO₄) in the hypothalamus were determined fluorometrically. Immobilization stress caused both significant decrease in NA level and increase in MHPG-SO₄ level in rats treated with saline, naloxone at 1 mg/kg and 5 mg/kg in comparison to controls. In spite of the finding that 5 mg/kg of naloxone by itself significantly reduced hypothalamic NA level, pretreatment with 5 mg/kg of the drug significantly enhanced both the decrease in NA level and increase in MHPG-SO₄ level induced by stress. Enhancement of the stress-induced increase in the hypothalamic NA turnover is believed to be due to blockade of opiate receptors by the drug. These results suggest that endogenous opioid peptides in the hypothalamus might be partially involved in stress process by attenuating the increase in NA turnover induced by stress.

Key words: immobilization stress — naloxone — noradrenaline — MHPG-SO₄ — endogenous opioids — hypothalamus

Introduction

A variety of stressful stimuli are generally accepted to activate the brain noradrenergic system in many species (Stone, 1975).

By simultaneously measuring levels of noradrenaline (NA) and its major metabolite, 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-SO₄) (Schanberg et al. 1968 a, 1968 b) considered a useful index of NA turnover in rat brain (Meek and Neff, 1973; Kohno et al. 1981),

we reported that NA turnover increases in specific areas of rat brain including the hypothalamus, amygdala, hippocampus, thalamus, pons plus medulla oblongata and cerebral cortex, after the animals have been exposed to immobilization stress (Tanaka et al. in press) or tail shock under immobilization stress (Nakagawa et al. 1981). We also noted time-related differences in increases in NA turnover induced by stress; in particular, the most rapid and marked increase in NA turnover is observed in the hypothalamus (Tanaka

et al. in press; Nakagawa et al. 1981). However, the significance of stress-induced increases in NA turnover remains controversial.

With the demonstration of endogenous opioid peptides (Hughes, 1975) and subsequent determination of several structures (Hughes et al. 1975; Simantov and Snyder, 1976; Li and Chung, 1976), numerous efforts have been made to clarify the roles of these peptides in the brain.

In addition, analgesia has been reported to follow stressful stimuli such as immobilization (Amir and Amit, 1978), foot shock (Akil et al. 1976; Madden et al. 1977) or cold water swim (Bodnar et al. 1978). This is believed to be due to opioids in the brain, since analgesia is attenuated by opioid antagonists such as naloxone.

Further, Rossier et al. (1977) and Guillemin et al. (1977) have demonstrated ACTH and β -endorphins increase concomitantly in the plasma of rats exposed to stress.

These findings suggest that opioids in the brain play a role in stress situations.

The present study investigates the effects of endogenous opioids by using their antagonist, naloxone (Martin, 1967) on stress-induced increases in NA turnover in the hypothalamus, which contains high concentrations of not only NA and MHPG-SO₄ (Kohno et al. 1981) but also endogenous opioids (Kobayashi et al. 1978; Barchas et al. 1978) and moderate densities of opiate receptors (Atweh and Kuhar, 1977). The hypothalamus is also the site of noradrenergic neurons which respond to stress more rapidly and markedly than in other areas of the brain (Tanaka et al. in press).

Methods

Male Wistar rats weighing 170-190 g were housed 4 to a cage (265×425×150 mm standard cage containing wood shav-

ings) in a 12 hour (7:00 a.m. to 7:00p.m.) light/dark cycled room at constant room temperature (24±1°C) and humidity (50±10%). Food and water were provided *ad libitum*.

Naloxone hydrochloride (the gift from Sankyo K.K.) was dissolved in physiological saline, then 1 mg/kg or 5 mg/kg, referring to the free base, was injected subcutaneously in a volume of 0.2 ml/100 g body weight.

Immobilization was employed as a stress procedure by enclosing animals in a flexible wire mesh (3×3 mm) initially formed into a cone and then bent to conform to the size of the individual rats.

Rats were allocated to one of the following six groups. Those in the first three groups were injected with saline or with naloxone at 1 mg/kg or 5 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 naloxone. The former three groups served as controls for the latter three stressed-groups.

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 simultaneously by our fluorometric method (Kohno et al. 1979).

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

Results

Results are shown in Table 1. Immobilization stress caused significant decreases in hypothalamic NA levels in saline, naloxone 1 mg/kg and 5 mg/kg groups, compared to their respective controls. Naloxone alone decreased NA levels in a dose-dependent manner and the decrease by the drug

TABLE 1

Effect of naloxone pretreatment on changes in noradrenaline (NA) and 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-SO₄) contents (ng/g) in the rat hypothalamus induced by immobilization stress

Treatment	NA	MHPG-SO ₄
Saline	2305.2±93.90	280.1±10.97
Saline + Stress	1739.4±60.44 ^{a***}	483.9±26.82 ^{a***}
Naloxone 1 mg/kg	2268.0±87.29	269.2±10.11
Naloxone 1 mg/kg + Stress	1658.1±70.55 ^{a***}	532.5±37.03 ^{a***}
Naloxone 5 mg/kg	1979.8±46.47 ^{b**}	254.4±10.55
Naloxone 5 mg/kg + Stress	1407.4±47.65 ^{a***} _{c***}	571.0±25.22 ^{a***} _{c*}

Each value indicates the mean±S.E.M. of 8 rats.

Statistical significance:

a: stress vs respective controls

b: vs saline control

c: vs saline+stress

Statistical significance levels are:

*P<0.05, **P<0.01, ***P<0.001

See text for detail.

at 5 mg/kg was statistically significant. The hypothalamic NA level in rats treated with 5 mg/kg of naloxone followed by stress was significantly reduced compared to levels in animals stressed after saline injection.

MHPG-SO₄ levels in the hypothalamus in saline, naloxone 1 mg/kg and 5 mg/kg groups were significantly increased by immobilization stress compared to levels in the respective controls. Although naloxone alone tended to decrease the metabolite levels, no statistical significance was obtained compared to saline controls. The hypothalamic MHPG-SO₄ level in stressed rats pretreated with naloxone at 5 mg/kg was significantly increased compared to that in stressed animals with saline.

Discussion

In the present study, immobilization stress caused both a significant decrease in NA level and an increase in MHPG-SO₄ level in the hypothalamus. This indicates

that release of NA is enhanced in the hypothalamus by immobilization stress, as previously reported by us (Tanaka et al. in press).

Naloxone at 5 mg/kg alone significantly decreased the hypothalamic NA level and tended to decrease the MHPG-SO₄ level, but not significantly. This seems to be due to an inhibitory action of the drug on catecholamine synthesis, as described by García-Sevilla et al. (1978).

In spite of the inhibitory effect of naloxone on catecholamine synthesis, the present study shows that pretreatment with naloxone at 5 mg/kg significantly enhanced the stress-induced increase in NA turnover in the hypothalamus; both the decrease in NA level and the increase in MHPG-SO₄ level caused by stress were significantly greater in rats pretreated with naloxone at 5 mg/kg than those with saline. Together with the findings that the hypothalamus contains high concentrations of opioids (Kobayashi et al. 1978; Barchas et al. 1978) and moderate densities of opiate receptors (Atweh and Kuhar,

1977), enhancement of the stress-induced increase in NA release in the hypothalamus by naloxone seems to be due to blockade of opiate receptors by the drug. These receptors are the binding sites of endogenous opioid ligands of which release might be increased during stress. The idea that endogenous opioids released during stress play a role in the stress process is supported by reports that ACTH and β -endorphins are concomitantly released into the blood of stressed rats (Rossier et al. 1977; Guillemin et al. 1977). Further support is found in reports which show that various stresses, including immobilization (Amir and Amit, 1978), cause analgesia, which can be attenuated by naloxone in rats (Akil et al. 1976; Bodnar et al. 1978) and in humans (Willer and Albe-Fessard, 1980), or are accompanied by changes in stereospecific bindings of tritiated naloxone (Madden et al. 1977) or etorphin (Chance et al. 1977) in the rat brain.

Taken together with our previous report which showed that the hypothalamus contains very high levels of both NA and MHPG-SO₄ and that noradrenergic activity in the hypothalamus increases more markedly and rapidly than in other regions examined, we suggest there is a close relationship between opioid peptide system and noradrenergic system in the hypothalamus, specifically that opioid peptides are released to provide a protective or inhibitory role against increases in NA release or excessive release of NA caused by stress. This is reasonable, since in cerebral cortex recent evidence indicates that opiate receptors are localized presynaptically on terminals of noradrenergic neurons (Llorens et al. 1978) and that stimulation of these receptors by morphine or β -endorphin results in inhibition of NA release from their nerve terminals (Abilla and Langer, 1978).

The hypothalamus plays an important role in the regulatory function of the autonomic nervous system, the endocrine

system, and emotion. Furthermore, naloxone is reported to induced tension-anxiety in human (Grevert and Goldstein, 1977) and to potentiate emotional responses in rats (Rodgers and Deacon, 1979; Green et al. 1979; File, 1980). Redmond et al. (1979) reported that a brain noradrenergic system might be involved in anxiety in monkeys. These data suggest that endogenous opioid peptides act to reduce emotion heightened by stress, i. e., to relieve anxiety or fear in animals exposed to noxious stimuli, in part, by attenuating the activity of noradrenergic neurons in the hypothalamus.

Multiple forms of opiate receptors have been proposed to be present in nervous tissue (Llord et al. 1977; Audigier et al. 1977; Terenius, 1977) and relatively higher concentrations of naloxone are required to antagonize effects mediated by some of these receptors (Lord et al. 1977). These studies suggest that the drug dosages used in our study are suitable as reported by Holtzman (1979) and adequate to block low affinity sites of these receptors (Audigier et al. 1977).

The assumption in the present study is that naloxone has no pharmacological actions other than those related to the blockade of opiate receptors. However, Sawynok et al. (1979) pointed out that naloxone might have pharmacological actions unrelated to opiate receptor blockade. Dingleline et al. (1978) reported that naloxone may be an antagonist of γ -aminibutyric acid (GABA). The present finding cannot be explained by the antagonistic action of naloxone to GABA, since such an action appears when much larger doses of the drug are applied than those used in the present study.

Barchas et al. (1978) suggested that endorphins might well play critical roles in behavioral and emotional responses to the environment, if these peptides were part of the basic systems which modulate responses to pain and stress.

The present study supports the view proposed by Barchas et al. (1978) and reveals, indirectly, that endogenous opioid peptides in the hypothalamus are partially involved in the stress process in this region. The idea is further supported by our recent finding using morphine (unpublished observation), even when actions of naloxone unrelated to the opioid peptide system are taken into consideration.

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