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Naloxone, Given Before But Not After Stress Exposure, Enhances Stress-Induced Increases in Regional Brain Noradrenaline Release

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TANAKA, M , Y IDA AND A TSUDA *Naloxone, given before but not after stress exposure, enhances stress-induced increases in regional brain noradrenaline release* PHARMACOL BIOCHEM BEHAV **29**(3)613–616, 1988 —Male Wistar rats were injected with either saline or naloxone at a dose of 5 mg/kg either 10 min before exposure to a 1-hour period of immobilization stress or after exposure to the same stress for 2 hours which was then followed by a further 1-hour stress exposure (a total of 3 hours of immobilization stress) Levels of noradrenaline (NA) and its major metabolite, 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-SO_4) in six discrete brain regions were determined fluorometrically Both one hour and three hours of immobilization stress significantly increased MHPG-SO_4 levels in all brain regions examined This effect was accompanied by significant reductions of NA levels excluding the cerebral cortex after 1 hour of stress Naloxone, injected prior to stress exposure, significantly enhanced MHPG-SO_4 increases in the hypothalamus, amygdala and thalamus, but did not do so when injected 2 hours after stress exposure Naloxone administration at either time did not affect stress-induced increases in MHPG-SO_4 levels in the hippocampus, cerebral cortex or pons plus medulla oblongata These results suggest that naloxone enhances stress-induced increases in NA release in the hypothalamus, amygdala and thalamus only during the early period of immobilization stress Furthermore, these findings suggest that endogenous opioid peptides might be preferentially released during the initial exposure to stress

Immobilization stress Naloxone Opioid peptides Noradrenaline release Rat brain regions

A variety of stressful stimuli have been known to increase noradrenaline (NA) release in extended brain regions in rats [10–17]

Previously, we reported that naloxone, an opioid antagonist, injected prior to stress exposure, enhances stress-induced increases in NA release in the hypothalamus, amygdala and thalamus [13] We further suggested that endogenous opioid peptides released during stress might act to attenuate stress-induced increases in NA release in these brain regions [13] This hypothesis has been partly supported by the findings that a potent opiate, morphine, and an opioid peptide, Met-enkephalin, injected prior to stress-exposure, significantly attenuate stress-induced increases in NA release in the hypothalamus and amygdala as well as in the hippocampus, pons plus medulla oblongata (pons+med.obl) and midbrain [15–17]

We have also reported that there exists a non-homogeneous regional brain time-course of NA release in response to immobilization stress [14] The brain regions where naloxone injected prior to stress exposure enhances stress-induced increases in NA release are the same regions wherein the most marked increases in NA release occur very rapidly, i e , within the first hour of stress [14] This raises the possibility that if naloxone was injected later, i e , at a certain time after exposure to stress, the drug would enhance increases in NA release in other brain regions In order to investigate this hypothesis, we examined the effects of naloxone, given at two different times, i e , 10 min prior to and 2 hours after stress exposure, on stress-induced increases in NA release in six brain regions, by measuring levels of NA and its major CNS metabolite, 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-SO_4), which is a valid index of NA release

METHOD

Male Wistar rats, weighing 170–190 g, receiving a standard diet (solid diet CE-2, Clea, Japan) with water freely available, were housed 4 to each cage (265×425×150 mm standard plastic cage containing wood shavings) in a 12 hr light/dark cycled room (light on at 0700 and off at 1900 hr) at a constant temperature (24±1°C) and humidity (50±10%)

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

Naloxone hydrochloride (a gift from Sankyo K.K.) was dissolved in physiological saline, and a 5 mg/kg dose of the drug (referring to the free base) was injected subcutaneously.

By balancing their body weights, the rats were allocated to five groups of eight animals each Rats in two groups were injected with either naloxone at a dose of 5 mg/kg or saline (2 ml/kg), 10 min before a 1-hour period of immobilization stress Animals in two other groups were injected with either naloxone or saline after exposure to immobilization stress for 2 hours and then continued to be immobilized for a

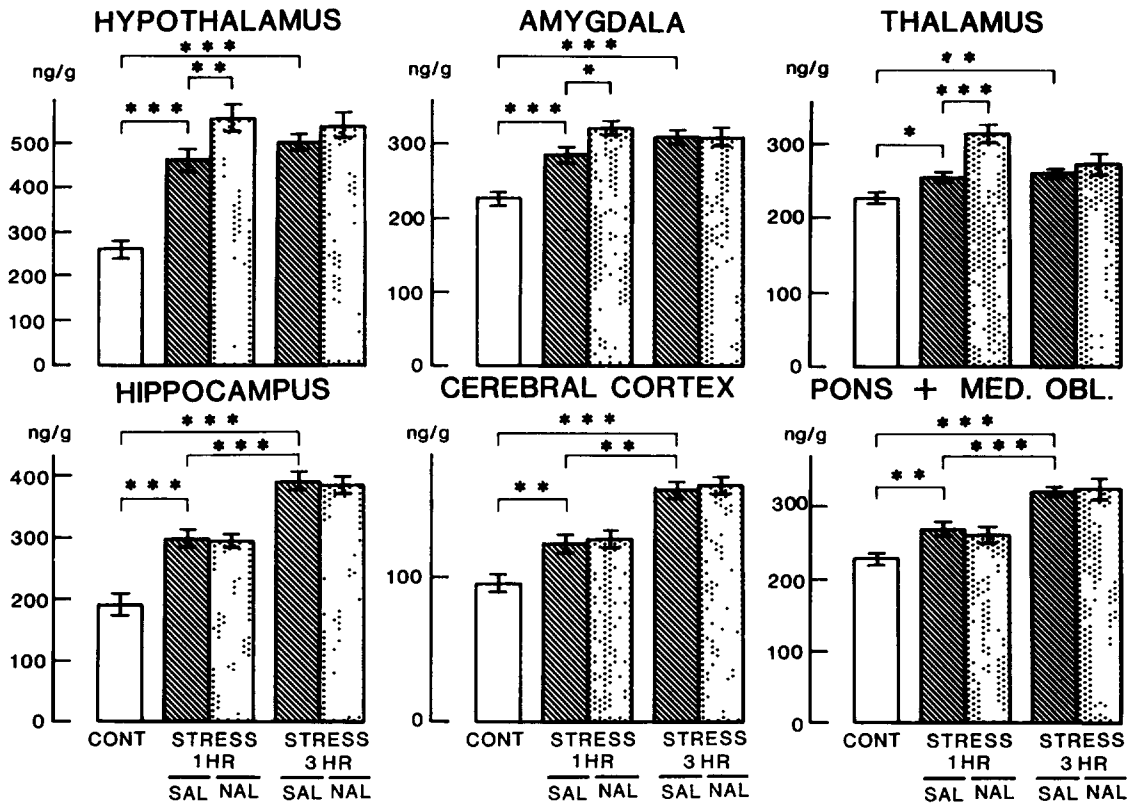

FIG 1 Effects of naloxone on changes in MHPG-SO_4 levels caused by immobilization stress Abbreviations are, SAL saline, NAL naloxone at 5 mg/kg Each value indicates the mean±S E M of 8 rats Statistical significance *$p<0$ 05, **$p<0$ 01, ***$p<0$ 001 See the text for details

further one hour period Untreated rats served as controls All experiments were carried out between 1000 hr and 1400 hr, since we found no diurnal variations of either NA or MHPG-SO_4 levels during this time period [7]

Immediately after each treatment, the rats were sacrificed by decapitation The brain was rapidly removed and dissected into discrete brain regions according to the method of Glspen *et al* [3] and frozen on solid CO_2 Brain regions dissected were hypothalamus, amygdala, thalamus, hippocampus, pons+med obl and cerebral cortex Brain tissues were stored at −45°C until assayed NA and MHPG-SO_4 levels in the brain regions were determined simultaneously by our fluorometric method [6]

For statistical analysis, Student's *t*-test (two-tailed) was employed.

RESULTS

Immobilization stress, both for 1 hour and for 3 hours, significantly increased MHPG-SO_4 levels in all brain regions examined as compared with those in control rats (Fig 1) These increases were accompanied by significant reductions in NA levels with the exception of the cerebral cortex in 1-hour stressed rats (Fig. 2)

Naloxone at a dose of 5 mg/kg injected prior to stress exposure significantly enhanced increases in MHPG-SO_4 levels caused by stress in the hypothalamus, amygdala and thalamus but not in the hippocampus, cerebral cortex or pons+med obl , as compared to saline-injected and post-stress-injected rats In contrast, the same dose of naloxone, administered 2 hours after stress exposure, did not enhance increases in the metabolite levels caused by stress in any brain regions examined

Reductions in NA levels caused by stress were significantly enhanced by naloxone injected prior to stress, only in the hypothalamus of 1-hour stressed rats No obvious changes were produced by naloxone injected either before or after stress exposure in other brain regions examined.

DISCUSSION

Immobilization stress for 1 hour and for 3 hours significantly increased MHPG-SO_4 levels in all brain regions examined and reduced NA levels in these regions, with the exception of the cerebral cortex of 1-hour stressed rats These results are consistent with many previous reports and suggest that immobilization stress increases NA release in extended brain regions of rats [10–17].

Naloxone, given before stress exposure, enhanced the reduction of NA levels only in the hypothalamus It is often observed that increases in MHPG-SO_4 levels do not always accompany reductions of NA levels depending upon the brain regions examined and experimental conditions used [12–15] In addition, naloxone is reported to mildly inhibit catecholamine synthesis [2], so that changes in the metabolite levels seem to be more indicative of NA release than those of the parent amine (NA)

The finding that naloxone at a dose of 5 mg/kg, injected 10

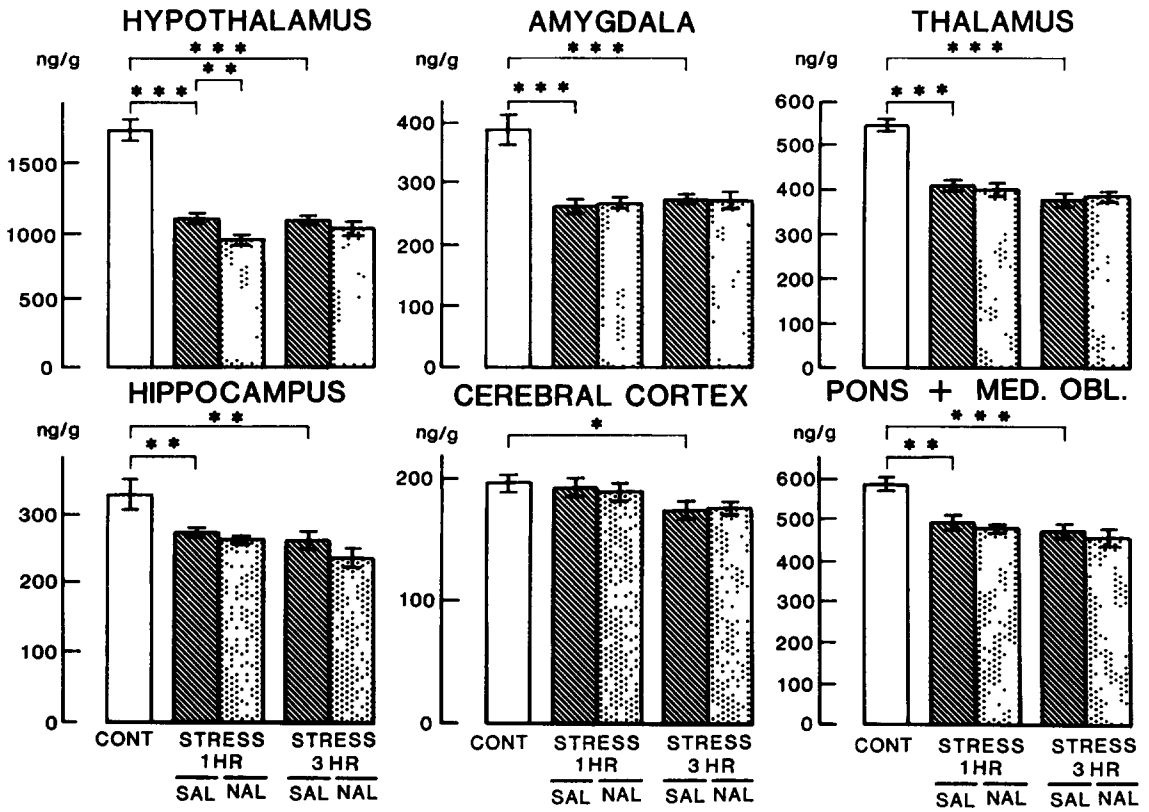

FIG 2 Effects of naloxone on changes in noradrenaline levels caused by immobilization stress Abbreviations are, SAL saline, NAL naloxone at 5 mg/kg Each value indicates the mean±S E M of 8 rats Statistical significance *$p<0$ 05, **$p<0$ 01, ***$p<0$ 001 See the text for details

min before stress exposure, enhances stress-induced increases in MHPG-SO_4 levels in the hypothalamus, amygdala and thalamus but not in the hippocampus, cerebral cortex and pons+med obl., is consistent with our previous report [13] Five mg/kg of naloxone might be considered a high dose for opiate receptor blockade, however, this dose was employed in the present study, since even higher doses are known to be needed for the blockade of δ receptors, which are the binding site of Met-enkephalin, one of the most important endogenous opioid peptides [4] Furthermore, this dose of naloxone was found to be effective for the enhancement of stress-induced increases in NA release in our previous study [13]

In contrast to naloxone given prior to stress, the same dose of the drug, when given 2 hours after stress exposure, failed to affect stress-induced increases in MHPG-SO_4 levels not only in the hippocampus, cerebral cortex and pons+med.obl., but also in the hypothalamus, amygdala and thalamus This finding indicates that naloxone, given before but not after stress exposure, enhances stress-induced increases in NA release in the hypothalamus, amygdala and thalamus It appears that the enhancing effect of naloxone on MHPG-SO_4 increase is observed only during the early period of immobilization stress but not in the later period and, furthermore, that this drug selectively influences brain regions such as the hypothalamus, amygdala and thalamus. This finding that naloxone, even when given 2 hours after stress exposure, could not enhance MHPG-SO_4 increases in the hippocampus, cerebral cortex and pons+med.obl , indicates that the enhancing effects of this drug are specific to these brain regions and are not indicative of regionally different characteristics of NA release related to the time-course of immobilization, which was previously reported by us [14].

It may be the case that the lack of naloxone-enhancement following stress exposure is due to the fact that NA release has reached a "ceiling " This possibility, however, is unlikely, since more marked NA release occurs when rats are exposed to more intense stress [10]

In summary, the present study revealed that naloxone enhances stress-induced increases in NA release in the early but not the late periods of immobilization stress As previously reported, this enhancement by naloxone seems to be due to its blockade of endogenous opioid receptors (binding sites of endogenous opioid ligands), the release of which has been reported to increase during stress [1, 8, 9] We suggest that the release of endogenous opioid peptides by stress occurs primarily during the early period of stress and that these peptides act to attenuate stress-induced increases in NA release during this period This suggestion is supported by the findings that the reduction of Met-enkephalin in the guinea pig hypothalamus caused by stress is observed only at 30 min but not at 1, 3, and 6 hours following exposure to immobilization stress [5] and that Met-enkephalin injected ICV prior to or 5 min after, but not 10 min after stress exposure, effectively attenuates stress-induced increases in NA release in the hypothalamus and amygdala [17]

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