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Abstract

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KEYWORDS: morphine, corticotropin-releasing factor(CRF), norepinephrine, dopamine, stress

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EFFECTS OF MORPHINE ON HYPOTHALAMIC CORTICOTROPIN-RELEASING FACTOR (CRF), NOREPINEPHRINE AND DOPAMINE IN NON-STRESSED AND STRESSED RATS

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Abstract. The effects of morphine on the hypothalamic corticotropin-releasing factor (CRF), norepinephrine (NE) and dopamine (DA) concentrations were investigated in non-stressed and stressed rats. Acutely administered morphine stimulated both the synthesis and release of CRF in the hypothalamus, thereby activating the pituitary-adrenocortical system in non-stressed rats, but inhibited the stress-induced CRF synthesis and ACTH-corticosterone secretion. Either a morphine or ether-laparotomy stress reduced NE and DA concentrations in the hypothalamus. A pretreatment with morphine inhibited the stress-induced reduction in the hypothalamic NE and DA concentrations, and induced a significant increase in the DA concentration. These observations suggest that hypothalamic NE and DA are involved in morphine-induced changes in hypothalamo-pituitary-adrenocortical (HPA) activity and that endogenous opiates have a role in regulating CRF secretion by interacting with hypothalamic biogenic amines.

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Since the isolation of the endogenous opioid peptides, enkephalins (1) and endorphins (2), much attention has been directed to their roles in regulating pituitary function. Grossman and Besser (3) have reported that endogenous opiates inhibit corticotropin-releasing factor (CRF)-ACTH secretion tonically via the noradrenergic pathway in man. On the other hand, Buckingham and Cooper (4) have reported that hypothalamic CRF secretion in rats was stimulated after acute systemic administration of morphine *in vivo* and after morphine or enkephalin administration *in vitro* in a CRF bioassay system. Recently, Vale and his co-workers (5, 6) isolated 41-residue ovine and rat CRF which permitted the development of a radioimmunoassay for CRF. The effects of opioids on the hypothalamo-pituitary-adrenocortical (HPA) system have not been investigated with this procedure.

Our previous studies using a rat CRF radioimmunoassay showed that an intra-third ventricular administration of morphine, methionine-enkephalin or its potent analog, D-Ala²-met-enkephalinamide (DALA) stimulated CRF-ACTH re-

lease in unanesthetized, freely moving rats with third-ventricular cannulas and cervical venous catheters (submitted for publication). However, it is unclear whether opioids regulate CRF secretion by acting directly on the CRF neurons in the hypothalamus or by interacting with hypothalamic monoamines to secrete CRF.

Therefore, in the present study, we examined the effects of morphine on the hypothalamic CRF, norepinephrine (NE) and dopamine (DA) concentrations in non-stressed and stressed rats in order to study the relationship between the hypothalamic CRF level and the responses of these catecholamines. We also discussed the interaction of endogenous opioid peptides and hypothalamic catecholamines in the control of CRF secretion.

MATERIALS AND METHODS

Animals. Male Wistar rats, weighing about 250g, were used. The rats were kept in a 12 h light/dark cycled animal room (lights on 0700-1900 h) at a suitable temperature with food and water *ad libitum* for at least one week before the experiment. Before the day of experimentation, they were moved to a quiet laboratory and allowed to become acclimatized to the room conditions. The experiments were performed in the daytime (1230-1500 h) to avoid diurnal variations.

Treatments. Test rats were injected intraperitoneally with morphine hydrochloride dissolved in 0.9 % w/v sodium chloride (2 mg/0.4 ml/100 g body weight) and control rats were injected with the corresponding volume of physiological saline. The rats were decapitated before the injection and 15, 30 and 60 min after the injection. One group of rats was treated with morphine hydrochloride and 30 min later exposed to an ether-laparotomy stress for 15 min to examine the effects of morphine on acute stress-induced changes in HPA activity and hypothalamic NE and DA. A control group of rats was treated with physiological saline and 30 min later exposed to the same stress for the same duration. Immediately after the rats were decapitated, the hypothalamus was removed, and truncanal blood was collected from each rat. The serum was stored frozen until the ACTH and corticosterone assay.

Tissue extraction for CRF assay. A block of hypothalamic tissue was obtained which included the median eminence and was rostrocaudally bounded by the optic chiasm and mammillary bodies, and dorsally by the anterior commissure. A sagittal section through the third ventricle at the midline divided the hypothalamus equally into the right and left halves. Halves of the hypothalamus were put into iced plastic tubes and stored frozen until extraction. The tissue samples were homogenized in 2 ml of a solution composed of 80 % acetone and 20 % 0.5 N HCl with an ultrasound sonicator. Ten microliters of each tissue homogenate was dried and stored for protein measurement by Lowry's method (7). The rest of the homogenate was centrifuged at 4,000 g at 4°C for 10 min, and the supernatant was transferred to another glass tube. Three ml of petroleum ether was mixed with the supernatant, and the mixture was centrifuged at 1,200 g at room temperature for 5 min. The lower layer was transferred to another tube and dried at 45°C under a stream of nitrogen gas. The dried extracts were stored at -20°C until assay for CRF.

Radioimmunoassay of CRF. The radioimmunoassay of rat CRF was performed by a previously reported method (8). The rat CRF antiserum was developed in our laboratory. Synthetic tyrosyl-rat CRF and rat CRF from Peptide Institute Inc. (Osaka, Japan) were used for the tracer and standard, respectively. Iodination of rat CRF and the subsequent RIA

were carried out by the same procedure used for the ovine CRF-RIA (9).

Tissue extraction for NE and DA assay. The other halves of the hypothalamus were quickly put into plastic tubes with 1 ml of 0.2M HClO₄ and stored frozen until extraction. After 50 ng/50 μ l of 3, 4-dihydroxybenzylamine (DHBA) (Sigma) was added to each tube as an internal standard, the tissue samples were homogenized in the ultrasound sonicator. Ten microliters of tissue homogenate from each tube was stored for protein measurement. The rest of the tissue homogenate was centrifuged at 10,000 g at 4°C for 10 min, and 200 μ l of the supernatant was transferred to another conical tube containing 50 mg Al₂O₃ (Alumina) and 1.5 ml of Tris-HCl buffer (1.0M Tris, 0.004 % EDTA-2Na, pH 8.6). After mixing and centrifugation, the supernatant was aspirated off, and the precipitate was washed 3 times with distilled water. Catecholamine-absorbed alumina was placed into an MF-I filter kit (with RC58 Membrane filter 0.2 μ m, Bioanalytical Systems Inc. USA) and centrifuged at 1,200 g for 2 min to extract the catecholamines from the alumina into the receiving tube. Twenty-five or fifty microliters of extracted sample was injected into the HPLC system.

Determination of NE and DA. NE and DA in the hypothalamus were determined by ion-pairing reverse-phase high performance liquid chromatography with amperometric detection. The chromatographic system was composed of : a pump, IRICA P-321; injector, IRICA U-350; column, Lichrosorb RP18 (5 μ m, 4 mm $\times$ 250 mm); amperometric detector, IRICA E-308 and recorder, HITACHI 056. The solvent consisted of 0.1 M KH₂PO₄, 0.5 mM EDTA-2Na and 100 mg/l sodium 1-heptane sulfonate, the 'ion-pairing' reagent. A flow rate of 1.5 ml/min, applied voltage of 0.75 V on the detector electrode and a strip chart speed of 5 mm/min were maintained. Under these conditions, the intraassay coefficient of variation was 9.2 %, and the interassay coefficient of variation was 12.8 %. The assay sensitivities of NE and DA were 10 pg and 15 pg, respectively.

Measurement of serum ACTH and corticosterone. ACTH was measured by radioimmunoassay using a commercially available RIA kit (ACTH Radioimmunoassay Kit, CEA-IRE-Sorin, France). Corticosterone was measured with 50 μ l of sample by radioimmunoassay using a cortisol RIA kit (SPAC Cortisol Radioimmunoassay Kit, Daiichi Radioisotope Labs, Tokyo, Japan). Nakane *et al.* (10) reported that the cross reactivity of this antiserum was 7.2 % to corticosterone, 3.5 % to 11-deoxycortisol and under 0.5 % to other steroids. As cortisol and 11-deoxycortisol are present at very low levels in rat blood, the measured corticosterone values are ascribed mostly to corticosterone. The values obtained by this method were compared with those by corticosterone-fluorescence spectrophotometry. The correlation between them was high ($r = 0.8554$, $p < 0.01$).

Statistical analysis. Statistical analysis was conducted by Duncan's new multiple range test.

RESULTS

Effect on CRF-ACTH-corticosterone activity

The hypothalamic CRF concentration tended to increase at 60 min after morphine administration, but the response was not statistically significant compared to the saline-treated group. The serum ACTH level was significantly elevated at 30 and 60 min, while the serum corticosterone level was significantly elevated from 15 min to 60 min after the administration compared to the saline-treated group (Fig. 1, Table 1).

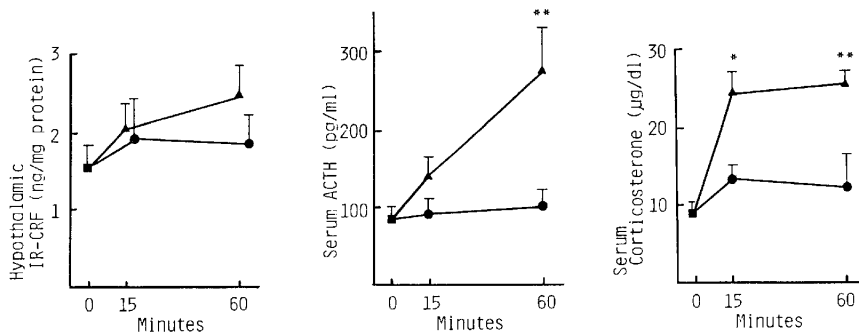


Fig. 1. Changes in hypothalamic CRF, serum ACTH and corticosterone concentrations after an intraperitoneal injection of morphine (2 mg/100 g b.w.) (▲) or saline (●). Each point and bar represents the mean and SEM of 6 determinations. * $p < 0.05$, ** $p < 0.01$ vs the level at each time point in the saline group.

TABLE 1. HYPOTHALAMIC CRF, SERUM ACTH AND CORTICOSTERONE CONCENTRATIONS IN INTACT RATS (CONTROL) AND RATS TREATED WITH SALINE ALONE, MORPHINE ALONE, SALINE PLUS STRESS AND MORPHINE PLUS STRESS

Treatment		IR-CRF (ng/mg protein)	ACTH (pg/ml)	corticosterone (μg/dl)
control	(n = 8)	1.54 ± 0.14	67.2 ± 13.3	1.7 ± 1.1
saline	(n = 6)	1.49 ± 0.15	91.4 ± 20.9	7.6 ± 2.9 ^j
morphine	(n = 6)	1.71 ± 0.19	372.4 ± 116.9 ^d	35.4 ± 4.4 ^e
saline + stress	(n = 7)	3.19 ± 0.62 ^a	455.4 ± 71.0 ^e	28.0 ± 2.3 ^h
morphine + stress	(n = 7)	0.92 ± 0.14 ^{b, c}	503.6 ± 87.7 ^f	28.3 ± 3.9 ⁱ

Values represent the mean and SEM.

^a $p < 0.01$ vs saline group, ^b $p < 0.01$ vs saline plus stress group, ^c $p < 0.01$ vs morphine group,

^{d, e, f, g, h, i} $p < 0.01$ vs saline group.

^j $p < 0.01$ vs control group, ^{d, e, f, g} not statistically significant compared to any other value.

Effect on acute stress-induced changes in CRF-ACTH-corticosterone activity

The hypothalamic CRF concentration, serum ACTH and corticosterone levels were significantly elevated 15 min after the onset of the ether-laparotomy stress in saline-pretreated rats compared to saline-treated, non-stressed rats. However, in morphine-pretreated, stressed rats, the hypothalamic CRF concentration was significantly reduced, and no further elevation of serum ACTH and corticosterone levels were observed compared to saline-pretreated, stressed rats or morphine-treated, non-stressed rats (Table 1).

Effect on hypothalamic NE and DA content

Both the NE and DA concentrations in the hypothalamus were significantly reduced in morphine-treated rats compared to saline-treated rats. Both the NE and DA concentrations in the hypothalamus were significantly reduced after 15

TABLE 2. HYPOTHALAMIC NOREPINEPHRINE AND DOPAMINE CONCENTRATIONS IN RATS TREATED WITH SALINE ALONE, MORPHINE ALONE, SALINE PLUS STRESS AND MORPHINE PLUS STRESS

Treatment		norepinephrine (ng/mg protein)	dopamine (ng/mg protein)
saline	(n = 5)	50.3 ± 3.5	12.8 ± 1.5
morphine	(n = 6)	40.2 ± 2.2 ^a	7.4 ± 0.8 ^c
saline + stress	(n = 5)	37.4 ± 1.1 ^b	7.6 ± 0.4 ^d
morphine + stress	(n = 6)	39.5 ± 2.0	12.6 ± 0.7 ^{e, f}

Values represent the mean and SEM.

^a, ^b, ^c, ^dp < 0.01 vs saline group, ^ep < 0.01 vs morphine group, ^fp < 0.01 vs saline plus stress group.

min exposure to the ether-laparotomy stress in saline-pretreated rats to the same degree as in morphine-treated, non-stressed rats. In morphine-pretreated rats, the exposure to the stress for the same duration did not induce a further reduction in the hypothalamic NE and DA concentrations, but induced a significant increase in the DA concentration compared to saline-pretreated, stressed rats or morphine-treated, non-stressed rats (Table 2).

DISCUSSION

In the present study, the authors investigated the effects of morphine on the CRF-ACTH-corticosterone activity and hypothalamic NE and DA contents in non-stressed and stressed rats. The systemic dose of morphine was decided on the basis of other studies (4, 11).

In this study, we observed a statistically insignificant tendency to increase for the hypothalamic CRF content 60 min after the morphine administration, and simultaneously observed a significant elevation in the serum ACTH and corticosterone levels. Also, in another study, we observed that an intra-third ventricular administration of 30 µg of morphine in unanesthetized, freely moving rats did not change the CRF content in the median eminence, but increased it significantly in the rest of the hypothalamus including the CRF-production site, paraventricular nuclei, and simultaneously elevated the plasma ACTH and corticosterone levels (submitted for publication). Considering these data, the result of the determinations of the hypothalamic CRF content in this study suggests that morphine acts on the hypothalamic area including the CRF-production site, stimulating the synthesis and release of CRF almost equally, and consequently activating the HPA system.

The serum corticosterone level was significantly elevated even 15 min after the morphine administration, while the serum ACTH level did not show a significant change at 15 min. This result suggests that morphine may act directly on the adrenal cortex as well as on the hypothalamus, as Lymangrover *et al.* (12) and Meites (13) have reported for naloxone and opioids. However, it dose not

seem that morphine acts directly on the anterior pituitary, since some investigators have reported that localized lesions of the hypothalamus block the effect of morphine (14) and that morphine has no effect on the pituitary *in vitro* (4).

Briggs and Munson (15) and Gibson *et al.* (16) reported that morphine inhibited stress-induced ACTH secretion. Inversely, Buckingham and Cooper (4) have reported that morphine exaggerated stress-induced CRF secretion in the hypothalamus and ACTH secretion from the anterior pituitary in their bioassay system, although they used adrenaline as a stressor. Our results agree with the reports by Briggs and Munson (15) and Gibson *et al.* (16) that morphine inhibited stress-induced CRF synthesis and ACTH-corticosterone secretion and that morphine plus stress reduced CRF synthesis more than morphine alone.

Morphine has been reported to increase NE turnover in the rat brain (17-19). Tanaka *et al.* (18) have also reported that morphine acts to attenuate the stress-induced increase in NE release in the hypothalamus, amygdala and thalamus via opiate receptors, while it facilitates NE release in these regions in non-stressed rats. According to our results, morphine or ether-laparotomy stress reduced the hypothalamic NE content, but pretreatment with morphine inhibited the stress-induced reduction in NE content. It is difficult to discuss the turnover rate of these catecholamines from our results, as changes in tissue content may reflect synthesis, storage, release or inactivation. Nevertheless, our results may indicate that morphine increases the NE release in the hypothalamus in non-stressed rats and inhibits the stress-induced increase in the NE release in the hypothalamus, in agreement with the report by Tanaka *et al.* (18).

Furthermore, morphine has been reported to increase DA turnover in the rat brain (20). However, some investigators have reported that the increase in the DA turnover by morphine or DALA is mainly an intraneuronal phenomenon, and consequently these opioids inhibit DA release from the nerve terminals, since both morphine and DALA do not increase the formation of 3-methoxytyramine (3-MT), a catabolite formed from the nerve terminals (21-23). This study showed that morphine or ether-laparotomy stress reduced the hypothalamic DA content, but that pretreatment with morphine abolished the stress-induced reduction in the DA content and induced a significant increase in the DA content. The stress-induced reduction in the DA level in the hypothalamus may indicate that DA release exceeds synthesis, although the stress may stimulate both DA release and synthesis. However, the morphine-induced reduction in the DA level may reflect the diminished activity of DA neurons, since morphine is considered to increase the intraneuronal DA turnover, thus decreasing DA release from the nerve terminals (21-23). These possible mechanisms may result in excess DA synthesis over release in morphine-pretreated, stressed rats, which in turn leads to the increase in the hypothalamic DA content.

Considering that acutely administered morphine stimulated the CRF-ACTH-corticosterone activity, but inhibited the stress-induced secretion of CRF, ACTH

and corticosterone, it is assumed that hypothalamic NE and DA are involved in morphine-induced changes in HPA activity and that endogenous opioid peptides have a role in regulating CRF secretion by interacting with hypothalamic biogenic amines. Further study measuring the turnover rate of amines is necessary to elucidate the interaction between endogenous opioid peptides and biogenic amines which regulate CRF secretion.

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REFERENCES

1. Hughes, J., Smith, T.W., Kosterlitz, J.W., Fothergill, L.A., Morgan, B.A. and Morris, H.R.: Identification of two related pentapeptides from the brain with potent opiate agonist activity. *Nature* **258**, 577-579, 1975.
2. Cox, B.M., Opheim, K.E., Teschemacher, H. and Goldstein, A.: A peptide-like substance from pituitary that acts like morphine. *Neuroendocrinology* **38**, 411-417, 1975.
3. Grossman, A. and Besser, G.M.: Opiates control ACTH through a noradrenergic mechanism. *Clin. Endocrinol.* **17**, 287-290, 1982.
4. Buckingham, J.C. and Cooper, T.A.: Differences in hypothalamo-pituitary-adrenocortical activity in the rat after acute and prolonged treatment with morphine. *Neuroendocrinology* **38**, 411-417, 1984.
5. Vale, W., Spiess, J., Rivier, C. and Rivier, J.: Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and β -endorphin. *Science* **213**, 1394-1397, 1981.
6. Rivier, J., Spiess, J. and Vale, W.: Characterization of rat hypothalamic corticotropin-releasing factor. *Proc. Natl. Acad. Sci. USA* **80**, 4851-4855, 1983.
7. Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J.: Protein measurement with Folin phenol reagent. *J. Biol. Chem.* **193**, 265-275, 1951.
8. Hashimoto, K., Hattori, T., Murakami, K., Suemaru, S., Kawata, Y., Kageyama, J. and Ota, Z.: Reduction in brain corticotropin-releasing factor (CRF) in spontaneously hypertensive rats. *Life Sci.* **36**, 643-647, 1985.
9. Hashimoto, K., Murakami, K., Ono, N., Kageyama, J., Aoki, Y., Takahara, J. and Ota, Z.: A specific radioimmunoassay for corticotropin releasing factor (CRF) using synthetic ovine CRF. *Life Sci.* **32**, 1001-1007, 1983.
10. Nakane, T., Kuwae, A., Watanabe, M. and Kanie, N.: Fundamental study and clinical application of SPAC cortisol kit. *Hormon to Rinsho* **28**, 193-197, 1980 (in Japanese).
11. Lotti, V.J., Kokka, N. and George, R.: Pituitary-adrenal activation following intrahypothalamic microinjection of morphine. *Neuroendocrinology* **4**, 326-332, 1969.
12. Lymangrover, J.R., Dokas, L.A., Kong, A., Martin, R. and Saffran, M.: Naloxone has a direct effect on the adrenal cortex. *Endocrinology* **109**, 1132-1137, 1981.
13. Meites, J.: Effects of opiates on neuroendocrine functions in animals: Overview. In *Opioid Modulation of Endocrine Function*, ed. G. Delitala, M. Motta and M. Serio, Raven Press, New York, pp. 53-63, 1984.
14. George, R. and Way, E.L.: The role of the hypothalamus in pituitary-adrenal activation and antidiuresis by morphine. *J. Pharmacol.* **125**, 111-115, 1959.
15. Briggs, F.N. and Munson, P.L.: Studies on the mechanism of stimulation of ACTH secretion with the aid of morphine as a blocking agent. *Endocrinology* **57**, 205-219, 1955.
16. Gibson, A., Ginsburg, M., Hall, M. and Hart, S.L.: The effects of opiate receptor agonists and

- antagonists on the stress-induced secretion of corticosterone in mice. *Br. J. Pharmacol.* **65**, 139-146, 1979.
17. Bloom, A.S., Dewey, W.L., Harris, L.S. and Brosius, K.K.: The correlation between antinociceptive activity of narcotics and their antagonists as measured in the mouse tail-flick test and increased synthesis of brain catecholamines. *J. Pharmacol. exp. Ther.* **198**, 33-41, 1976.
18. Tanaka, M., Kohno, Y., Tsuda, A., Nakagawa, R., Ida, Y., Iimori, K., Hoaki, Y. and Nagasaki, N.: Differential effects of morphine on noradrenaline release in brain regions of stressed and non-stressed rats. *Brain Res.* **275**, 105-115, 1983.
19. Borrell, J. and Borrell, S.: Brain catecholamines and adrenocortical activation by morphine in the cat. *Neurosci. Letters* **4**, 191-195, 1977.
20. Johnson, J.C., Ratner, M., Gold, G.J. and Clouet, D.H.: Morphine effects on the levels and turnover of catecholamines in rat brain. *Res. Commun. Chem. Pathol. Pharmacol.* **9**, 41-53, 1974.
21. Kuscinsky, K. and Hornykiewicz, O.: Morphine catalepsy in the rat: Relation to striatal dopamine metabolism. *Eur. J. Pharmacol.* **19**, 119-122, 1972.
22. Groppetti, A., Algeri, S., Cattabeni, F., DiGiulio, A.M., Galli, C.L., Ponzio, F. and Spano, P.F.: changes in specific activity of dopamine metabolites as evidence of a multiple compartmentation of dopamine in striatal neurons. *J. Neurochem.* **28**, 193-197, 1977.
23. Calderini, G., Consolazione, A., Garattini, S. and Algeri, S.: Different effects of methionine-enkephalin and (D-Ala²) methionine-enkephalin amide on the metabolism of dopamine and norepinephrine in rat brain: fact or artifact? *Brain Res.* **146**, 392-399, 1978.