Effect of Morphine on Hypothalamic Corticotropin-Releasing Factor (CRF) and Pituitary-Adrenocortical Activity

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Abstract

The effects of intraperitoneal and intra-third ventricular administration of morphine on the hypothalamic corticotropin-releasing factor (CRF) and the pituitary-adrenocortical activity were examined in unanesthetized, freely moving rats. Hypothalamic CRF was measured by rat CRF radioimmunoassay.

Intraperitoneal or intra-third ventricular administration of morphine increased blood concentrations of ACTH and corticosterone while intraperitoneal administration tended to increase CRF concentration in the whole hypothalamus including the median eminence and intra-third ventricular administration increased CRF concentration in the hypothalamus excluding the median eminence. However, morphine seemed to inhibit the increase in CRF concentration in the hypothalamus induced by the ether-laparotomy stress. The main site of morphine action on the hypothalamo-pituitary-adrenocortical system seemed to be in the hypothalamic area.

After Nasmyth's (1954) article on adrenal ascorbic acid depletion by morphine, many investigators have reported on the effects of morphine on the pituitary-adrenal system. Since the isolation of the endogenous opioid peptides, enkephalins and endorphins (Hughes *et al.*, 1975; Cox *et al.*, 1975), much attention has been directed to their roles in regulating pituitary function. Grossman and Besser (1981) have reported that opioids inhibit corticotropin-releasing factor (CRF)-ACTH secretion tonically via the noradrenergic pathway in man. On the other hand, Buckingham and Cooper (1984) have reported that hypo-

thalamic CRF secretion in rats was stimulated after acute systemic administration of morphine *in vivo* and after morphine or enkephalin administration *in vitro* in the CRF bioassay system. Recently, Vale *et al.* (1981) and Rivier *et al.* (1983) isolated 41-residue ovine and rat CRF, which made possible the development of a radioimmunoassay for CRF. The effects of opioids on the hypothalamo-pituitary-adrenocortical (HPA) system have not been determined by radioimmunoassay of CRF.

In the present study, we examined the effects of acute intraperitoneal and intra-third ventricular administration of morphine on the HPA system in unanesthetized, freely moving rats, using rat CRF radioimmunoassay.

Materials and Methods

Animals

Male Wistar rats, weighing 250–320 g, were kept in a 12 hr 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. The experiments were performed at the same time of day between 1230 h and 1500 h.

Experiment 1

Rats were injected intraperitoneally with either morphine hydrochloride dissolved in 0.9% w/v sodium chloride (2 mg/0.4 ml/100 g body weight) or the corresponding volume of physiological saline as controls. The rats were decapitated before, 15 and 60 min after injection.

Experiment 2

The rats were separated into 5 groups to examine the effects of morphine on acute stress-induced change in the HPA activity. One group of rats was decapitated without any treatment as the control rats. Two groups of rats were decapitated 30 min after intraperitoneal injection of physiological saline or morphine. Another two groups of rats were treated with physiological saline or morphine and 30 min later exposed to the ether-laparotomy stress for 15 min, and then decapitated.

In *Experiment 1* and *2*, immediately after decapitation, the hypothalamic tissue, including the median eminence, was removed and was bounded by the optic chiasm and mammillary bodies rostrocaudally and by the anterior commissure dorsally (Glowinski and Iversen, 1966). The truncal blood from each rat was collected into a chilled glass tube. The serum was frozen and stored until ACTH and corticosterone assay.

Experiment 3

Twenty-three gauge stainless steel cannulas were implanted into the third ventricles of rats (Antunes-Rodrigues and McCann, 1970). One week later catheters for collecting blood samples were inserted into the jugular veins. The next day, 3 μ g or 30 μ g of morphine hydrochloride in a 3 μ l volume of vehicle consisting of hydrochloride in distilled water (pH 3.5, the same

solution as the morphine solution) was injected into the third ventricle of unanesthetized, freely moving rats. The doses were selected on the basis of other studies (Lotti *et al.*, 1969; Buckingham and Cooper, 1984). Control rats were injected with 3 μ l of the vehicle. Seven hundred microliters of blood samples were drawn off over approximately 30 sec using heparinized syringes from the jugular catheters into chilled tubes and were replaced with the same amount of saline before, 10, 20 and 40 min after morphine injection. At 45 min after injection, the rats were decapitated. Immediately after decapitation, the median eminence area and the remaining tissue of the hypothalamus were dissected out on dry ice considering the difference between their CRF concentrations. First, the median eminence area was dissected, the size of which was about 2 mm in diameter and 0.4 mm in thickness, and then the rest of the hypothalamus was dissected, of which the borders were the optic chiasm rostrally, the mammillary bodies caudally and the horizontal line through the anterior commissure dorsally (Glowinski and Iversen, 1966). The blood samples were immediately centrifuged and the plasma was frozen. The plasma ACTH and corticosterone assay were carried out in a couple of days.

Tissue extraction for CRF assay

The hypothalamic tissues were placed into iced plastic tubes and stored frozen until extraction. Extraction was carried out as soon as possible to avoid the degradation by tissue enzymes (Hashimoto *et al.*, 1985, b). They were homogenized with an ultrasound sonicator in a 2 ml solution composed of 80% acetone and 20% 0.5 N HCl. Ten microliters of each tissue homogenate were dried and stored for protein measurement by Lowry's method (Lowry *et al.*, 1951). 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, and 3 ml of petroleum ether was added. They were then mixed and centrifuged at 1,200 g at room temperature for 5 min, and 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 CRF assay. The recovery rate of CRF during this extraction procedure was $79.9 \pm 4.2\%$, and the procedure was appropriate for CRF extraction (Hashimoto *et al.*, 1985, a).

Radioimmunoassay of CRF

The radioimmunoassay of rat CRF was performed by a previously reported method (Hashimoto *et al.*, 1985, a). 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 tracer and standard, respectively. Iodination of rat CRF and subsequent RIA were carried out by the procedure used for ovine CRF-RIA (Hashimoto *et al.*, 1983).

Measurement of plasma or 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). Corticosterone was diluted with the steroid free serum and used for the standard preparation (Suemaru *et al.*, 1985). Nakane *et al.* (1980) 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 from corticosterone-fluorescence spectrophotometry. The correlation between them was high ($r=0.8554$, $p<0.01$). This kit was used in the present investigation because of its simplicity.

Statistical analysis

Statistical analysis was conducted by Student's *t*-test.

Results

Experiment 1. Effects of intraperitoneal morphine administration on CRF-ACTH-corticosterone activity

Hypothalamic CRF concentration tended to increase at 60 min after administration of morphine but the response was not statistically significant. Serum ACTH level was significantly elevated at 60 min. Serum corticosterone level was also significantly elevated from 15 min to 60 min. (Fig. 1).

Fig. 1. Changes in hypothalamic CRF (CRF in the whole hypothalamus including the median eminence), serum ACTH and corticosterone concentrations after an intraperitoneal injection of morphine (2 mg/100 g b.w.). Injections of morphine ($\blacktriangle$) and saline ($\bullet$) are shown. 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.

Fig. 2. Hypothalamic CRF (CRF in the whole hypothalamus including the median eminence), serum ACTH and corticosterone concentrations in rats treated with the saline alone

Experiment 2. Effects of intraperitoneal morphine administration on acute stress-induced changes in CRF-ACTH-corticosterone activity.

Hypothalamic CRF concentration, serum

Fig. 3. CRF concentrations in the median eminence area (ME) and the remaining tissue of the hypothalamus except the median eminence (RHV) at 45 min after intra-third ventricular administration of morphine. Each column and horizontal line represents the mean and SEM. The vehicle group (n=7), 3 μg morphine group (n=5) and 30 μg morphine group (n=5). *p<0.05.

(□), saline plus stress (▤), morphine alone (▨) and morphine plus stress (▧). The values in the intact control rats are shown with the column (□). Each column and horizontal line represents the mean and SEM. The number in the column indicates the number of rats used. *p<0.05, **p<0.01. (stress: ether-laparotomy stress).

ACTH and corticosterone levels were significantly elevated at 15 min after the onset of ether-laparotomy stress in saline-pretreated rats compared with the saline-treated, non-stressed rats. In morphine-pretreated, stressed rats, hypothalamic CRF concentration was significantly reduced and no further elevation of serum ACTH and corticosterone levels were observed compared with saline-pretreated, stressed rats or morphine-treated, non-stressed rats (Fig. 2).

Experiment 3. Effects of intra-third ventricular morphine administration on CRF-ACTH-corticosterone activity

No significant changes were found in the median eminence CRF concentration in the vehicle, 3 μ g morphine and 30 μ g mor-

phine injected groups at 45 min after administration. In the rest of the hypothalamus, CRF concentration was significantly higher in the 30 μ g morphine-injected group than the vehicle-injected group (Fig. 3).

The plasma ACTH level in the 3 μ g morphine-injected group was significantly elevated at 40 min after administration. In the 30 μ g morphine-injected group the plasma ACTH level was markedly elevated from 10 min to 40 min compared with the vehicle-injected group (Fig. 4).

The plasma corticosterone level in the 3 μ g morphine-injected group was significantly elevated at 20 min and 40 min. In the 30 μ g morphine-injected group, the plasma corticosterone level was significantly elevated from 10 min to 40 min compared with the vehicle-injected group (Fig. 5).

Fig. 4. Changes in plasma ACTH concentrations after intra-third ventricular administration of morphine in unanesthetized, freely moving rats. Each point and bar represents the mean and SEM. The vehicle group (n=7), 3 μ g morphine (n=5) and 30 μ g morphine (n=5). * p <0.05, ** p <0.01 vs the level at each time point in the vehicle group.

Fig. 5. Changes in plasma corticosterone concentrations after intra-third ventricular administration of morphine in unanesthetized, freely moving rats. Each point and bar represents the mean and SEM. The vehicle group (n=7), 3 μ g morphine (n=5) and 30 μ g morphine (n=5). * p <0.05, ** p <0.01 vs the level at each time point in the vehicle group.

Discussion

In the present study, morphine was microinjected into the third ventricle in addition to the intraperitoneal (systemic) administration to examine its direct effect on the hypothalamic level. Intraperitoneally administered morphine can act on each level of the HPA system: the hypothalamus, pituitary and adrenal glands, as it can pass through the blood brain barrier. On the other hand, morphine injected into the third ventricle can act not only on the hypothalamic tissue but partly enter the primary capillary plexus in the median eminence and reach the anterior pituitary via the portal vessels to act directly on the anterior pituitary cells. However, an intra-third ventricular administration is expected to have a more direct effect on the paraventricular region in the hypothalamus, which is a production site of CRF, than a systemic or a lateral-ventricular administration, since an administered substance is less diluted and more directly diffuses into the hypothalamic tissue. Lotti *et al.* (1969) reported a pituitary-adrenal activation following intrahypothalamic microinjection of morphine. But it is possible that an intrahypothalamic injection evokes a kind of stress reaction and activates the pituitary-adrenal system. Therefore, we used an intra-third ventricular administration as a better method of localized morphine administration (Antunes-Rodrigues and McCann, 1970).

We did not find a statistically significant change in the CRF concentration in the whole hypothalamus including the median eminence after administering morphine intraperitoneally. However, after intra-third ventricular administration of 30 μ g of morphine, the CRF concentration did not change in the median eminence area which was considered to be the CRF-releasing site, while the CRF concentration was significantly increased in the rest of the hypo-

thalamus. Since the paraventricular nuclei are considered to be the CRF-production site (Sawchenko and Swanson, 1985), it is suggested that acute administration of morphine stimulates the synthesis of CRF in the hypothalamus. The failure to detect a decrease in CRF concentration in the median eminence after morphine administration seems consistent with the findings of Buckingham (1984) that a single injection of morphine increased CRF content in the hypothalamus *in vivo* and that morphine increased both the CRF release and the hypothalamic CRF content *in vitro* using the CRF bioassay. It is not likely that changes in the CRF concentration in the median eminence is a sensitive index for the release of CRF into the portal blood.

Our present results do not neglect the possibility that morphine has a direct effect on the anterior pituitary corticotrophs. However, as several lines of evidence (George and Way, 1959; Lotti *et al.*, 1969; Gibson *et al.*, 1979; Buckingham and Cooper, 1984) show, it is assumed that the opiate has no direct action on the pituitary gland. Recently, we also have found that (D-al², met⁵)-enkephalinamide, a potent methionine-enkephalin analog, has no direct effect on the anterior pituitary corticotrophs in pituitary cell cultures (in submission).

As morphine or opioid peptides act on the hypothalamic monoamines, catecholamines (Kuscinsky and Hornykiewicz 1972; Borrell and Borrell 1977; Suemaru *et al.*, 1985) and serotonin (Algeri *et al.*, 1978; Van Loon and De Souza, 1978), morphine-induced changes in the hypothalamic CRF secretion may be via changes in the hypothalamic monoamines. Furthermore, it is also possible that morphine-induced changes in other ACTH secretagogues (i. e. amines, vasopressin etc.) stimulate ACTH secretion at the pituitary level.

On intraperitoneal administration, the serum corticosterone level was significantly elevated even at 15 min after administra-

tion, although the serum ACTH level did not show a significant change at 15 min. This result obtained by systemic administration may not negate the direct effect of morphine on the adrenal cortex, as Lymangrover *et al.* (1981) and Meites (1984) have reported that opioids, as well as naloxone, may act directly on the adrenal cortex as well as on the hypothalamus.

Briggs and Munson (1955) and Gibson *et al.*, (1979) reported that morphine inhibited the stress-induced ACTH secretion. On the other hand, Buckingham and Cooper (1984) have reported that morphine exaggerated the stress-induced CRF secretion in the hypothalamus and the ACTH secretion from the anterior pituitary in their bioassay system. In our study, if a significant reduction in the hypothalamic CRF concentration in "morphine plus stress" group is due to an excess release of CRF, the ACTH and corticosterone levels must be significantly elevated compared to "morphine alone" and "saline plus stress" groups, as the ACTH and corticosterone levels observed in "morphine alone" and "saline plus stress" groups are not the maximum levels in rats. However, no significant differences were observed between the serum ACTH and corticosterone values for these groups. These results suggest that the hypothalamic CRF reduction in the "morphine plus stress" group is not due to an excess release but due to an inhibition of CRF production.

In summary, our study suggests that an acute administration of morphine stimulates the synthesis of CRF in the hypothalamus, and that, on the other hand, morphine inhibits the CRF production in the hypothalamus induced by the ether-laparotomy stress. The main site of morphine action on the HPA system seems to be in the hypothalamic area.

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