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Diurnal alteration in opiate effects on the hypothalamo-pituitary-adrenal axis: changes in the mechanism of action

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Abstract

Opioid control of the hypothalamo-pituitary-adrenocortical axis has a characteristic circadian rhythm (Kiem, Kanyicska, Stark and Fekete, 1987). To elucidate the mechanisms leading to circadian alterations of opioid control of the hypothalamo-pituitary-adrenocortical axis, and to look for the receptor type at which the p.m. inhibitory action of opioids occurs, we examined the effect of morphine at different doses and the interaction between naloxone and morphine at different times of day in intact male Wistar rats. In the morning: morphine (10 and 30 mg/kg s.c.) significantly increased corticosterone secretion, while 3 mg/kg s.c. had no effect. Naloxone in a dose of 2.5 mg/kg i.p. significantly antagonized the corticosterone-releasing effect of morphine, suggesting that the secretion of corticosterone induced by morphine is mediated via μ-opioid receptors. In the afternoon: basal plasma corticosterone levels were higher than those in the morning, and morphine caused a significant corticosterone increase only at the dose of 30 mg/kg, and had no effect in the dose of 10 mg/kg. Morphine significantly decreased corticosterone levels in the dose of 3 mg/kg. This inhibitory action lasted approximately 3 h after morphine injection and was able to inhibit the circadian evening rise of corticosterone. The effect of morphine and the interaction between naloxone and morphine on prolactin secretion remained unchanged from a.m. to p.m.; naloxone (2.5 mg/kg i.p.) which inhibited the 30 mg/kg morphine-induced corticosterone rise in the morning failed to antagonize the 3 mg/kg morphine-induced decrease of corticosterone secretion in the afternoon. A high dose of naloxone (10 mg/kg i.p.) effectively prevented the 3 mg/kg morphine-induced p.m. inhibition of corticosterone secretion. The results of this study point to dual mechanism(s) of opioid action on the hypothalamo-pituitary-adrenal axis: the a.m. stimulatory mechanism is naloxone-sensitive, while the p.m. inhibitory mechanisms is naloxone-resistant.

Keywords: Diurnal alteration; Opioid mechanism; ACTH (adrenocorticotrophin); Corticosterone; Prolactin

1. Introduction

The nature of the opioidergic control of the hypothalamo-pituitary-adrenal axis might be different in various species: in humans, only the inhibitory action of morphine has been demonstrated (Allolio et al., 1987; Grossman et al., 1986; Taylor et al., 1986), whereas in conscious and non-stressed rats morphine exerts a stimulatory effect on the adrenocorticotropin (ACTH)/corticosterone secretion (Buckingham, 1982; Buckingham and Cooper, 1984; Buckingham and Cooper, 1986), however, high doses of this drug are required (Nikodjevic and Maickel, 1967; Harracz et al., 1981). There is evidence that in humans morphine is able to inhibit the circadian morning rise of cortisol (McDonald et al., 1959). Recently, we have demonstrated in the rat that the opioid action on the hypothalamo-pituitary axis has a characteristic circadian rhythm. Morphine stimulates ACTH/corticosterone secretion in the morning, whereas, in the afternoon when the baseline plasma corticosterone level is higher than in the morning, the morphine action is inhibitory on the secretion of these hormones with doses smaller than 10 mg/kg body weight (Kiem et al., 1987). In the rat, the stimulatory effect of morphine on the ACTH/corticosterone is antagonized by naloxone

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(Buckingham, 1982; Buckingham and Cooper, 1986), suggesting that it is mediated via μ-opioid receptors. This antagonizing effect of naloxone has, however, not yet been demonstrated for the p.m. inhibitory action of morphine. To elucidate the mechanisms leading to circadian alterations of opioid control on ACTH/corticosterone secretion, and to look for the receptor type at which the p.m. inhibitory action of morphine occurs, we examined the effect of morphine at different doses and the interaction of naloxone and morphine on ACTH/corticosterone secretion in the morning and in the afternoon.

2. Materials and methods

Male CFY Sprague-Dawley rats weighing 250–300 g were used throughout the study. The animals were housed three to five per cage at 23–25°C and 50–60% humidity with a 12/12 h light/dark (light on 06:00, off at 18:00) cycle. The rats were given rat chow and tap water ad libitum, and were handled once daily for at least 3–5 consecutive days preceding the experiments. One day before the experiments, the rats were weighed and housed two per cage. The experimental room was closed for 18 h before the experiment and only the two experimenters were present during blood collection. Blood samples were collected by decapitation 30 min after morphine injection or via an indwelling i.v. cannula (placed 2 days before the experiments) at intervals of 0 and 30, 60 and 120 min after morphine injection (Experiment 3). All experiments were performed in randomized groups of conscious and unrestrained animals.

Morphine sulphate (Alkaloida, Tiszavasvari, Hungary) was dissolved in saline and injected at the doses of 3, 10 and 30 mg/kg s.c.; naloxone (Du Pont) was dissolved in saline and injected at the dose of 2.5 and 10 mg/kg i.p. 15 min before morphine injection.

Interaction between naloxone and morphine, effect on corticosterone secretion: naloxone at the doses of 2.5 or 10 mg/kg was i.p. injected first, and 15 min later morphine (3, 10 and 30 mg/kg s.c.) was administered as second drug. Experiments were performed at 08:00–10:00 a.m. and 16:00–18:00 p.m. To demonstrate p.m. specificity of the morphine action on the corticosterone secretion, plasma prolactin concentration was also measured in the same plasma samples as corticosterone in this experimental set up.

To examine the effect of morphine on the p.m. rise of corticosterone, a dose of 3 mg/kg was subcutaneously injected into the free-moving cannulated rats at 16:00 p.m. and blood samples were collected at intervals of 30, 60 and 120 min after morphine injection.

Hormone measurement: the plasma corticosterone concentration was measured by radioimmunoassay. The antiserum was raised in rabbit against corticosterone-carboxymethyloxime-bovine serum albumin. ^{125}I-labelled corticosterone-carboxymethyl-oxime-tyrosine-methyl ester was used as tracer. Assay characteristics: sensitivity, 0.15–80 pmol/ml; interassay variation, 10%; intraassay variation, 5.5%; sensitivity, 0.2 fmol/ml. The plasma prolactin concentration was measured by radioimmunoassay, using materials kindly supplied by the NIDDK Rat Pituitary Hormone Distribution Program. Assay characteristics were sensitivity 2 ng/ml, interassay variation 15%, intraassay variation 9%.

Statistical analysis was done using logarithmic transformation of the data by two-way analysis of variance (ANOVA) followed with Dunnett's test for multiple comparison.

3. Results

In the morning, morphine at doses of 10 and 30 mg/kg significantly increased corticosterone and pro-

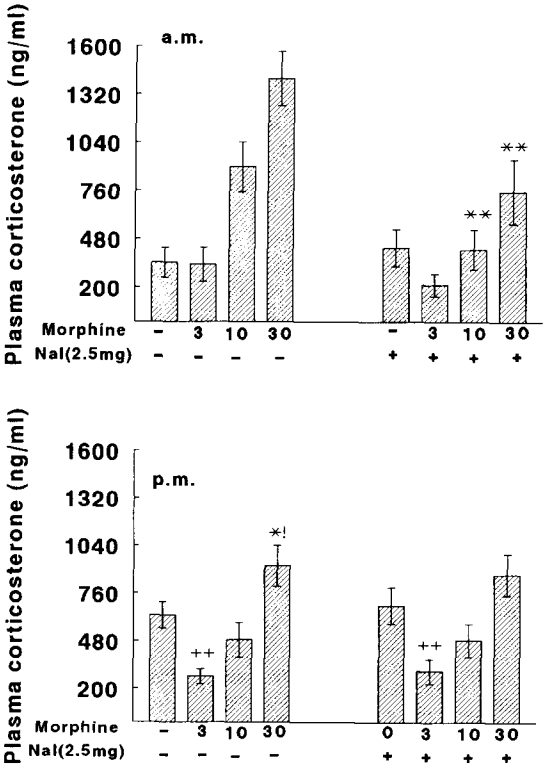

Fig. 1. Effect of morphine (3, 10 and 30 mg/kg s.c.) interaction with naloxone (2.5 mg/kg i.p.) on plasma corticosterone secretion in the morning (Fig. 1 a.m.; $n = 10$) and in the afternoon (Fig. 1 p.m.; $n = 18$). All values are the means ± S.E.M.; ** $P < 0.01$ compared to the corresponding values for morphine-treated animals, without naloxone. * $P < 0.05$ compared to basal corticosterone levels. $^{++}$ $P < 0.01$ compared to basal corticosterone levels.

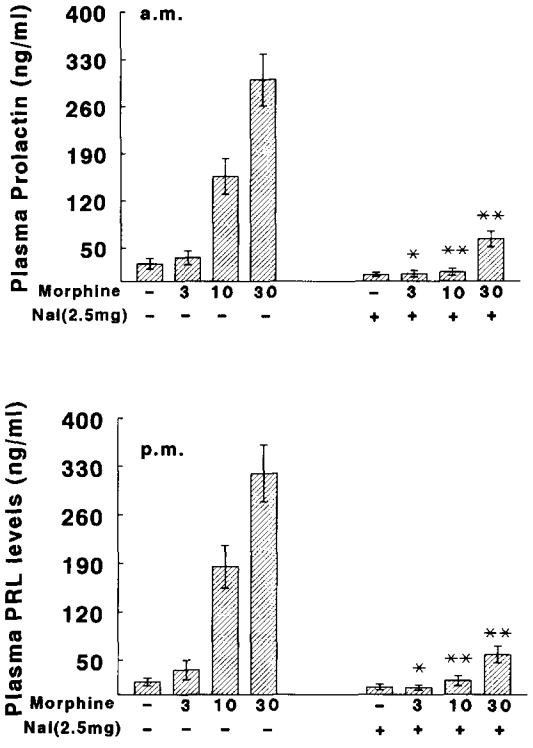

Fig. 2. Effect of morphine (3, 10 and 30 mg/kg s.c.) interaction with naloxone (2.5 mg/kg i.p.) on plasma prolactin release in the morning (Fig. 2 a.m.; $n = 10$) and in the afternoon (Fig. 2 p.m.; $n = 18$). The same plasma samples as in Fig. 1. All values are the means ± S.E.M.; $^{*}P < 0.05$, $^{**}P < 0.01$ compared to the values for morphine-treated, without naloxone.

lactin secretion. However, 3 mg/kg morphine had no effect on corticosterone and prolactin secretion as compared to the values for saline-treated animals (Fig. 1 a.m. and Fig. 2 a.m.). Naloxone in a dose of 2.5 mg/kg significantly antagonized the corticosterone and prolactin releasing effect of morphine (Fig. 1 a.m. and Fig. 2 a.m.), suggesting that the plasma corticosterone and prolactin secretion induced by morphine in the morning is mediated via μ-opioid receptors.

In the afternoon, when baseline plasma corticosterone levels were higher than those in the morning, morphine caused a significant corticosterone secretion only at the high dose of 30 mg/kg. Morphine slightly decreased plasma corticosterone levels at a dose of 10 mg/kg which stimulated corticosterone secretion in the morning. Interestingly, 3 mg/kg morphine, which had no effect on the a.m. corticosterone secretion, significantly inhibited corticosterone secretion in the afternoon (Fig. 1 p.m.). This inhibitory action lasted approximately 3 h after morphine injection and was able to inhibit the circadian evening rise of corticosterone (Fig. 3). In contrast to corticosterone, the morphine-induced prolactin secretion remained unchanged in the afternoon (Fig. 2 p.m.). The sensitivity to naloxone was also changed in the afternoon. Naloxone, 2.5

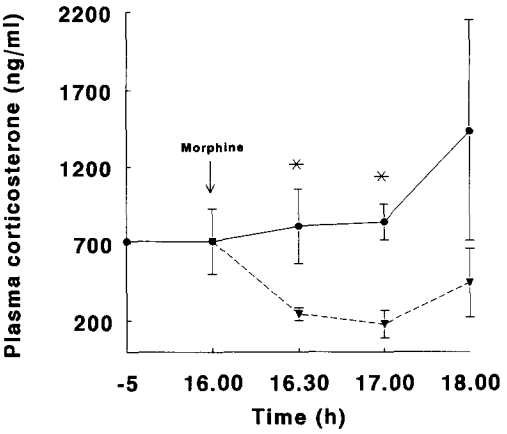

Fig. 3. Morphine (3 mg/kg s.c. triangles) was able to inhibit the circadian evening rise of corticosterone. This inhibitory effect of morphine lasted approximately 3 h after drug injection. Morphine injection at 16:00 p.m. Blood samples were collected via i.v. cannula ($n = 6$). $^{*}P < 0.05$ compared to the corresponding values for morphine-treated animals (filled circles).

mg/kg, which was enough to inhibit the 30 mg/kg morphine-induced corticosterone secretion in the morning, failed to antagonize the 3 mg/kg morphine-induced decrease in corticosterone secretion in the afternoon (Fig. 1 p.m.), whereas it fully antagonized the p.m. prolactin-releasing effect of morphine (Fig. 2 p.m.). The high dose of 10 mg/kg naloxone effectively prevented the 3 mg/kg morphine-induced p.m. inhibition of corticosterone secretion (Fig. 4), implicating that the p.m. decrease in corticosterone secretion induced by morphine is not mediated via naloxone-sensitive opioid receptors.

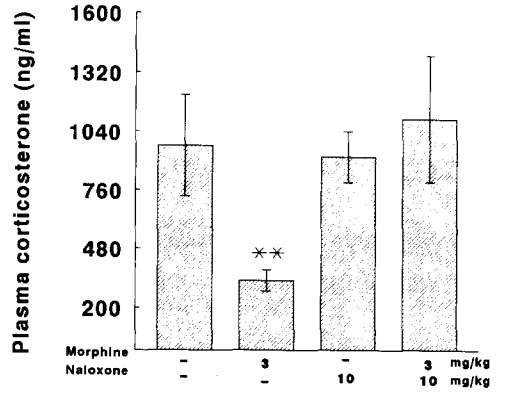

Fig. 4. Effect of morphine (3 mg/kg s.c.) and its interaction with 10 mg/kg naloxone which was injected 15 min before morphine on the corticosterone secretion in the afternoon; 10 mg/kg naloxone fully prevented the 3 mg/kg morphine-induced p.m. decrease of corticosterone secretion. $^{**}P < 0.01$ compared to the values for saline-, naloxone-, and naloxone-morphine-treated animals; $n = 5$–6 per group.

4. Discussion

Alterations in the mechanism of the morphine actions on the hypothalamo-pituitary axis are represented by dual effects of morphine on the hypothalamo-pituitary-adrenal axis, stimulating ACTH/corticosterone secretion at high doses (10–30 mg/kg s.c.) in the morning and inhibiting the secretion of these hormones at a low dose (3 mg/kg s.c.) in the afternoon. The a.m. stimulatory mechanism(s) of morphine is (are) naloxone-sensitive, while the p.m. inhibitory mechanism(s) is (are) naloxone-resistant. This (these) latter mechanism(s) might be of physiological importance in the opioid modulation of the hypothalamo-pituitary-adrenal axis since it appears parallel with the time of the peak in corticotrophin-releasing factor (CRF) content in the hypothalamus and maximal secretion of ACTH from the pituitary and of corticosterone from the adrenal glands in the rat, which peaks around 17:00–18:00 p.m. (Dallman et al., 1978; Guillemin et al., 1959; Nicholson et al., 1985).

The necessary doses for inhibition are significantly smaller than those required for stimulation. Furthermore, the morphine-like inhibitory action on the hypothalamo-pituitary-adrenal axis has also been observed with the endogenous opioid peptides, β-endorphin (Plotsky, 1986), and [Met^5]enkephalin (Del Pozo et al., 1980; Tsagarakis et al., 1990).

The nature of the effect and the mechanisms whereby morphine inhibits the p.m. hypothalamo-pituitary adrenocortical activity in the rat are not clear. However, it is generally accepted that morphine and endogenous opioid peptides influence hypothalamo-pituitary-adrenocortical activity by stimulating (Buckingham, 1982; Buckingham and Cooper, 1986; Cover and Buckingham, 1989) or inhibiting (Allolio et al., 1987; Buckingham and Cooper, 1984; Cover and Buckingham, 1989; Tsagarakis et al., 1989; Tsagarakis et al., 1990) via specific opioid receptors in the hypothalamus controlling CRF secretion. This is confirmed by the results of in vitro studies (Buckingham, 1982) which demonstrated the failure of morphine and opioid peptides to stimulate directly ACTH secretion from the isolated pituitary. The inhibitory action of morphine (Tsagarakis et al., 1989; Tsagarakis et al., 1990) and/or high concentrations of β-endorphin (Buckingham, 1986) on CRF-41 release has been demonstrated in vitro. Morphine and/or β-endorphin directly inhibited the release of stimulated CRF-41 from rat hypothalamus, and this inhibitory action was reversed by high doses of naloxone or by the κ-opioid antagonist MR2266.

There is a close anatomical relationship between opioid and CRF localization. Hypothalamic prodynorphin is mostly synthesized in neurons of the paraventricular nucleus (Cod and Fallon, 1986), where it is co-localized with CRF and vasopressin (Watson et al., 1982). The presence of nerve terminals containing β-endorphin (Finley et al., 1981) and dynorphin (Palkovits et al., 1983), and directly or indirectly impinging on irCRF-positive perikarya has been demonstrated (Roth et al., 1989).

The anatomical relationship between α-adrenergic (Liposits et al., 1986) and GABA-ergic (Vincent et al., 1982) neurons to CRF-synthesizing neurons has been reported. Furthermore, it was demonstrated that adrenergic (Ganong, 1977) and GABA-ergic (Makara and Stark, 1974) systems may tonically inhibit the secretory action of hypothalamo-pituitary-adrenal axis. Hence, the inhibitory action of morphine on ACTH/corticosterone secretion might also be due to the interaction between morphine and these neurotransmitter systems.

According to the present results, the selective interaction between morphine and naloxone that controls ACTH/corticosterone secretion is mediated by μ-opioid receptors (Buckingham and Cooper, 1984; Buckingham and Cooper, 1986; Cover and Buckingham, 1989) and is only observed in the morning. This interaction is lost in the afternoon, parallel with the appearance of the inhibitory action of morphine on ACTH/corticosterone secretion, showing that the p.m. decrease of corticosterone secretion induced by morphine is not mediated via μ-opioid receptors or is not due to a specific opioid action. This suggestion is based on the fact that naloxone, the selective μ-opioid receptor antagonist, at the dose of 2.5 mg/kg, which is high enough to antagonize the corticosterone secretion induced by a maximal dose of 30 mg/kg morphine in the morning, failed to prevent the decrease of corticosterone levels induced by only 3 mg/kg morphine in the afternoon.

A high dose of naloxone (10 mg/kg s.c.) has the ability to reverse the p.m. inhibitory action of morphine on corticosterone secretion. This result is consistent with those of Tsagarakis and co-workers (1990), and Buckingham (1986) who reported that the suppression of the in vitro stimulated CRF release induced by morphine and other opioid peptides was only reversed with high doses of naloxone or the κ-opioid receptor antagonist MR2266. This is also consistent with the results obtained in humans (Allolio et al., 1987).

Jacobson and Wilkinson (1986) recently reported the existence of diurnal variation in the binding of the opioid antagonist, [^{3}H]naloxone, to slices of the mediobasal hypothalamus, and the ability of naloxone to release LH is highest in the morning and reaches a nadir in the afternoon. Taking these results together with our present in vivo results, we suggest that the mechanism changing morphine actions on the hypothalamo-pituitary adrenocortical axis from a.m. to p.m. might be associated with changes in opioid receptor

subtypes. The p.m. inhibitory action of morphine on ACTH/corticosterone secretion might be related to specific opioid receptors located in the hypothalamus, possibly via κ or σ receptors (naloxone-insensitive receptors) as postulated (Allolio et al., 1987; Cover and Buckingham, 1989).

The physiological significance of the p.m. inhibitory action of morphine on the hypothalamo-pituitary-adrenal axis in the rat as described here is still unclear. However, it has been reported that morphine has the ability to inhibit the circadian morning cortisol rise (McDonald et al., 1959) and blocks the cortisol response to surgical stress (George et al., 1974) in humans and decreases the corticosterone response to ether stress (Suemaru et al., 1989) in the rat. We now demonstrated the inhibitory action of this drug on the p.m. ACTH/corticosterone secretion, which appears parallel with the time of maximal activity of the hypothalamo-pituitary adrenocortical axis. Thus, it seems that opioid peptides acting via specific opioid receptors (naloxone-insensitive receptors) participate in the mechanism(s) of the adaptation process. We suggest that the inhibitory mechanism of opioids protects the system from using up its entire reserve under conditions when cortisol or corticosterone levels are high.

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