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Changes in hypothalamic paraventricular nucleus catecholaminergic activity after acute and chronic morphine administration

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Abstract

The participation of hypothalamic noradrenaline in the expression of neuroendocrine signs of morphine withdrawal has been proposed. The present study in rats examined: (1) the relationships between corticosterone secretion and the possible modifications in noradrenaline and dopamine content and turnover in the hypothalamic paraventricular nucleus after acute and chronic morphine administration; (2) the changes in cyclic adenosine monophosphate (cAMP) levels in the paraventricular nucleus after the same treatments. The results showed that acute morphine injection in control rats increased corticosterone release, 3-methoxy-4-hydroxyphenylethylene glycol (MHPG) production, and noradrenaline turnover. Dopamine turnover in the paraventricular nucleus was decreased and the cAMP levels remained unchanged. In chronic morphine-treated rats, there was no elevation in noradrenaline turnover or in corticosterone secretion, indicating that tolerance developed to the acute effects of the opioid. Correspondingly, no alterations in dopamine turnover were observed when chronic morphine-treated rats were compared with control rats acutely injected with morphine. cAMP levels in the paraventricular nucleus were unchanged during the tolerant state. The results raise the possibility that noradrenergic afferents play a significant role in the alterations of paraventricular nucleus function and pituitary–adrenal axis activity in response to acute and chronic morphine and suggest that these modifications are not mediated through adenylate cyclase activation. The present data provide further support for the idea of adaptive changes in noradrenergic neurons projecting to the paraventricular nucleus during chronic morphine exposure. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Noradrenaline; Dopamine; Hypothalamus–pituitary–adrenocortical axis; Tolerance; Morphine; Paraventricular nucleus

1. Introduction

The repeated use of opioids induces adaptive changes in the nervous system leading to the development of tolerance and dependence. Acutely administered opioids potently stimulate the hypothalamic–pituitary–adrenal axis, which involves the activation of opioid receptors (Buckingham and Cooper, 1984). Thus, morphine and other opioid agonists increase adrenocorticotropin (ACTH) release, which in turn elevates glucocorticoid secretion. Chronic exposure to opioids results in the development of tolerance to their effects on endocrine secretion, as well as in physical dependence characterised by an increased hypothalamic–pituitary–adrenocortical function (Ignar and Kuhn, 1990). Recent results from our laboratory have shown a relationship between the activity of the hypothalamus–pituitary–adrenocortical axis and the functional responsiveness of the corticotrophin-releasing factor (CRF) and vasopressin systems in the hypothalamic paraventricular nucleus and median eminence after chronic exposure of rats to morphine (Milanés et al., 1997). In addition, it has been demonstrated that intracerebroventricular administration of β-endorphin increases the expression of CRF messenger in the paraventricular nucleus of the rat, which suggests that the actions of opioids on the hypothalamus–pituitary–adrenocortical axis are mediated through CRF secretion (Wang et al., 1996).

The exact neurocircuitry involved in the effects of opioids on the HPA axis has not been clarified yet. Different studies have proposed that the secretion of ACTH and the release of corticosterone after acute administration of

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opioids are not due to direct effects on hypothalamic CRF neurons in the paraventricular nucleus or on pituitary corticotrophs. Rather, they result from an indirect effect of drugs acting through catecholaminergic neurons that project to the hypothalamus (Suemaru et al., 1989). Thus, noradrenaline content and turnover studies in the whole hypothalamus show that hypothalamic noradrenergic neurons are activated after acute morphine administration (Milanés et al., 1997). In addition, antagonists of noradrenergic receptors block the effects of morphine on hypothalamus–pituitary–adrenocortical axis activity (Martínez-Piñero et al., 1994).

The activity of the axis is regulated by a number of neurotransmitters systems, including noradrenaline. CRF neurons in the paraventricular nucleus are innervated by numerous afferents from other brain regions as well as by intrahypothalamic interneurons (Palkovits, 1987). This nucleus receives a dense noradrenergic innervation originating from brainstem structures, notably the A_2 group, the A_1 group in the ventrolateral medulla, and, to a lesser extent, the locus coeruleus (A_6) (Cunningham and Sawchenko, 1988). In addition, it has been proposed that noradrenaline interacts with the parvocellular division of the paraventricular nucleus (Owens and Nemeroff, 1991).

The abundant noradrenergic innervation of the paraventricular nucleus suggests an important role of brain noradrenaline in the regulation of CRF release, although the precise role of this neurotransmitter in the regulation of CRF secretion remains controversial. Consequently, both stimulatory and inhibitory actions of noradrenaline on CRF secretion have been proposed (Plotsky et al., 1989), although recent studies have suggested that noradrenaline stimulates CRF gene expression and CRF secretion, thus regulating positively the hypothalamus–pituitary–adrenocortical axis (Itoi et al., 1994). Dopamine has also been proposed to be implicated in the control of hypothalamic and pituitary hormone secretion, although the projections and functions of many hypothalamic dopaminergic cell groups are poorly understood (Everitt et al., 1992). Major intracellular signalling systems, including the cAMP-dependent protein kinase A and protein kinase C pathways, have been implicated in the regulation of CRF synthesis and/or release (Emanuel et al., 1990).

Several animal models have been used in order to investigate the mechanisms involved in the responsiveness to opioids and in the development of tolerance and dependence (Nestler et al., 1993). Many compensatory mechanisms have been described in the locus coeruleus following chronic opioid administration, including up-regulation of the cAMP system as well as alteration of gene expression (Guitart et al., 1992). The catecholaminergic system plays an important role in the development and maintenance of opioid dependence and in the effects upon drug withdrawal, as has been shown in several neuronal models such as the locus coeruleus and the nucleus accumbens (Nestler, 1992).

Several lines of evidence suggest that activation of the hypothalamus–pituitary–adrenocortical axis may be an important factor determining stress-induced vulnerability to drugs of abuse (Torres and Horowitz, 1996). The aims of the present study were (1) to elucidate the possible adaptive changes of noradrenergic and dopaminergic systems projecting to the paraventricular nucleus upon chronic morphine administration. For this, we measured noradrenaline and dopamine content and turnover in the rat hypothalamic paraventricular nucleus following acute and chronic morphine administration and compared them with the corticosterone response; and (2) the modulation of cAMP formation in the paraventricular nucleus in response to morphine treatment.

2. Materials and methods

Adult male Sprague–Dawley rats (200–230 g at the beginning of treatment) were housed three to four per cage under a 12-h light/dark cycle in a room with controlled temperature ($22 \pm 1°C$) and humidity ($50 \pm 10\%$); food and water were available ad libitum.

2.1. Experimental treatment

On the basis of previous studies (Gonzálvez et al., 1994), rats were rendered tolerant to morphine by s.c. implantation of morphine base pellets (75 mg), one on day 0, two on day 2 and three on day 4, under light ether anaesthesia. This treatment results in a profound state of tolerance/dependence (Milanés et al., 1997). Control animals were implanted with placebo pellets containing lactose on the same time schedule. On day 7 animals were treated acutely with saline i.p. or morphine HCl (30 mg/kg i.p.) and killed 30 min later, and analytical studies were conducted. This time was chosen since both the maximal behavioural and hypothalamus–pituitary–adrenocortical axis activating effect of opioids were seen at this point time in previous studies (Alcaraz et al., 1993). There were the following experimental groups for catecholamines, cAMP and corticosterone determination in the paraventricular nucleus and plasma: (1) chronic placebo (naive)-acute saline i.p.; (2) chronic placebo-acute morphine i.p.; (3) chronic morphine (tolerant)-acute saline i.p.; and (4) chronic morphine (tolerant)-acute morphine i.p. The weight gain of the rats was checked during treatment to ensure that morphine was liberated correctly from the pellets, since it is known that chronic morphine treatment induces a decrease in body weight gain due to a lower caloric intake (Berhow et al., 1995). To check that rats did not show weight loss, which is a characteristic symptom of opioid withdrawal, body weight was measured immediately prior to saline or morphine injection and 30 min later, immediately before killing.

2.2. Measurement of plasma corticosterone levels

Plasma corticosterone levels were used as a sensitive marker of hypothalamus–pituitary–adrenocortical axis activity (Yokoe et al., 1988). To minimise circadian variability, all experiments were performed between 9:00 and 10:00 h and the animals were decapitated between 10:00 and 10:45 h. Trunk blood was collected into ice-cooled tubes containing 5% EDTA and then centrifuged (2500 rpm; 4°C; 15 min). Plasma was separated, divided into two aliquots, and stored at −30°C until assayed for corticosterone. Plasma levels of corticosterone were estimated in two aliquots of each sample using a commercially available kit for rats (^{125}I-corticosterone radioimmunoassay, ICN, Biomedicals, USA). The sensitivity of the assay was 0.40 ng/ml. Inter- and intra-assay coefficients of variation were 6.5% and 4.4%, respectively. The antibody cross-reacted 100% with corticosterone and $<0.5\%$ with other steroids.

2.3. Estimation of catecholamines and their metabolites in the paraventricular nucleus

After decapitation, brains were removed, fresh-frozen, and stored immediately at −80°C until use. The hypothalamic tissue containing the entire paraventricular nucleus was dissected from one coronal brain slice according to Palkovits' technique (Palkovits, 1973), and the paraventricular nucleus corresponds to those in plates 25–26 (Palkovits and Brownstein, 1988). Noradrenaline, its metabolite in the central nervous system 3-methoxy-4-hydroxyphenylethylene glycol (MHPG), dopamine, and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) were determined by high-performance liquid chromatography with electrochemical detection (HPLC/ED). Bilateral tissue samples were weighed, placed in 600 μl cold perchloric acid (0.1 M), and homogenised with a Polytron-type homogeniser (setting 4 for 30 s). The homogenates were then centrifuged (15 000 rpm; 4°C; 15 min) and the supernatants were taken for analysis and filtered through 0.22 μm GV filters (Millipore). Two aliquots of the supernatant from the same tissue sample were used, the first for analysis of noradrenaline, dopamine and DOPAC and the second for MHPG analysis. From the first aliquot of each sample, 10 μl was taken and injected into a 5-μm C_{18} reverse-phase column (Waters) through a Rheodyne syringe-loading injector 200 μl loop. Electrochemical detection was accomplished with a glassy carbon electrode set at a potential of +0.65 V vs. the Ag/AgCl reference electrode (Waters). The mobile phase consisted of a 95:5 (v/v) mixture of water and methanol with sodium acetate (50 mM), citric acid (20 mM), 1-octyl-sodium sulphonate (3.75 mM), di-*n*-butylamine (1 mM), and EDTA (0.135 mM), adjusted to pH 4.3. The flow rate was 0.9 ml/min and chromatographic data were analysed with Millennium 2010 Chromatography Manager (Millipore) equipment. DOPAC, noradrenaline and dopamine were simultaneously detected by the described HPLC method at elution times of 3.40, 4.35 and 12 min, respectively. Under these conditions, MHPG was not observed. Since most of MHPG in the rat CNS is present in a sulphate conjugate form, the method for the determination of total MHPG in the paraventricular nucleus is based on the acid-catalysed hydrolysis of MHPG-sulphate (Artigas et al., 1986). The aliquots for MHPG analysis were kept in polypropylene, screw-capped tubes for 5 min in a water bath at 100°C. Tubes were then cooled on ice and centrifuged (4000 rpm; 4°C; 10 min). The supernatant of the hydrolysed samples was injected (50 μl) into the HPLC equipment. The eluent for MHPG determination was as described above, but without 1-octyl-sodium sulphonate. Under these conditions, MHPG eluted at 4.80–5 min. Noradrenaline, dopamine and their respective metabolites were quantified by reference to calibration curves run at the beginning and the end of each series of assays. Linear relationships were observed between the amount of standard injected and peak heights measured. The content of noradrenaline, MHPG, dopamine and DOPAC in the paraventricular nucleus was expressed as ng/g wet weight of tissue.

2.4. Determination of cAMP in the paraventricular nucleus

Levels of cAMP were determined by radioimmunoassay (^{125}I-TME-S-cAMP, Diagnostic Pasteur, France) following the indications of the manufacturer. After dissections, the paraventricular nucleus was weighed, homogenised in 600 μl cold perchloric acid (0.3 M) with a Polytron homogeniser and centrifuged (12 000 rpm, 15 min, 4°C). Supernatants were treated with potassium phosphate until pH 6.2 was reached. The sensitivity of the assay was 2 pmol/ml. Intra- and inter-assay coefficients of variation were 7.7% and 8.2%, respectively. The antibody cross-reacted 100% with 3′,5′-cAMP and less than 0.3% with other nucleotides.

2.5. Drugs and chemical

Pellets of morphine base (Alcaliber Labs., Madrid, Spain) or lactose were prepared by the Department of Pharmacy and Pharmaceutic Technology (School of Pharmacy, Granada, Spain). Noradrenaline bitartrate, MHPG hemipiperazinium salt, dopamine HCl and DOPAC (used as HPLC standards) were purchased from Sigma (St. Louis, MO). Morphine HCl (Alcaliber Labs., Madrid, Spain) was prepared fresh every day, dissolved in sterile 0.9% NaCl (saline) and injected in volumes of 0.15 ml/100 g body weight. Other reagents were of analytical grade.

2.6. Statistical analysis

The data are expressed as means ± S.E.M. Relative concentrations of noradrenaline, MHPG, dopamine and

DOPAC are expressed as percent changes from values for placebo-treated control rats. Data were evaluated by two-way analysis of variance followed by the Neuman–Keuls test. Non-paired Student's *t*-test was used when comparing the mean body weight changes. Analysis of correlation (Pearson correlation) was used to analyse the relationship between the two variables, MHPG/noradrenaline ratio and corticosterone concentration. Significance level was taken as $P < 0.05$.

3. Results

Rats rendered tolerant to morphine had a significantly ($P < 0.001$) lower body weight gain on day 7 (19 ± 2.2 and 15 ± 2.0 g) than the groups treated with placebo pellets (51 ± 1.0 and 45 ± 2.2 g). There was no significant change in body weight-loss 30 min after saline (i.p.) or morphine (30 mg/kg i.p.) injection in tolerant rats (3.1 ± 0.7 and 3.7 ± 0.8 g, respectively) when compared to the naive groups also receiving an injection of saline or morphine (3.9 ± 0.4 and 4.4 ± 0.6 g, respectively), indicating the absence of abstinence on the day of the experiment. In addition, 7 days after the beginning of morphine pellet implantation, rats did not show any of the behavioural or physical signs usually observed during opioid withdrawal. These results mean that the pelleting method provides continuous exposure to morphine from day 0 to the day of the experiment.

3.1. Effects of morphine administration on plasma corticosterone concentration

In naive rats, stimulation of opioid receptors by systemic administration of morphine (30 mg/kg i.p.) elicited a significant ($P < 0.001$) increase in plasma corticosterone levels measured 30 min later (Fig. 1; control level, 202.8 ± 12.6 ng/ml). Rats rendered tolerant to morphine did not show any significant change in corticosterone release when measured 30 min after saline injection, as compared with the saline-treated naive group (development of tolerance). After acute injection of morphine to morphine-pelleted rats on day 7 plasma corticosterone levels were significantly ($P < 0.001$) lower than those measured in naive rats also injected with the opioid (expression of tolerance; Fig. 1).

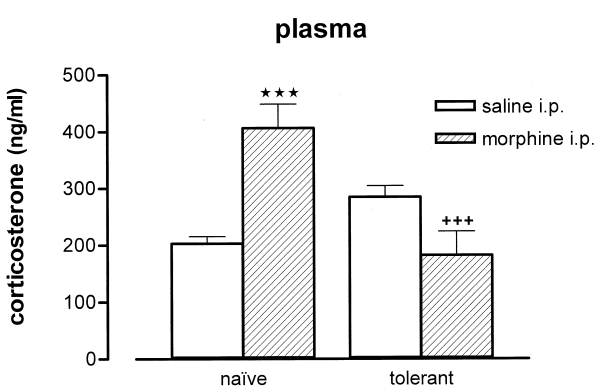

Fig. 1. Plasma corticosterone concentration in naive and tolerant rats, 30 min after acute injection of saline i.p. or morphine (30 mg/kg i.p.). Each bar represents the means ± S.E.M. ($n = 5$–8 per group). $^{***}P < 0.001$ vs. naive group injected with saline i.p.; $^{+++}P < 0.001$ when compared with naive group injected acutely with morphine.

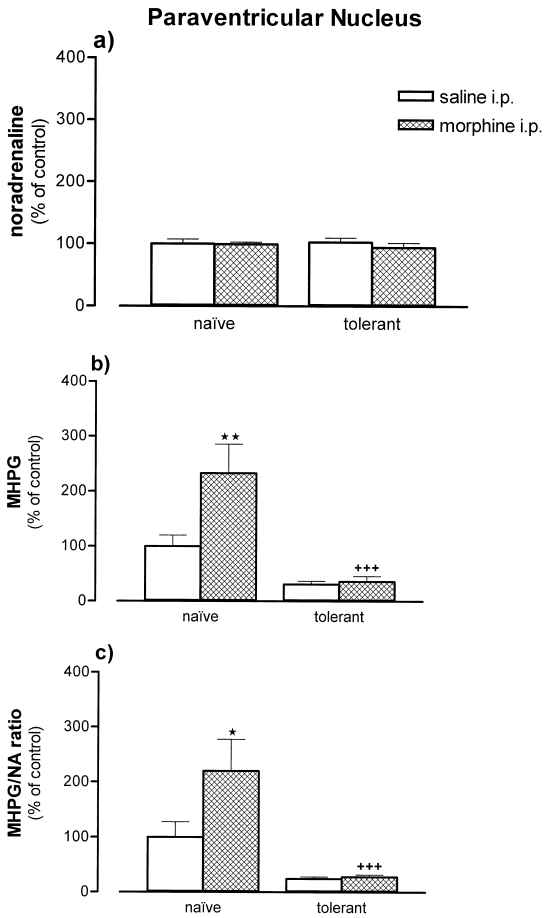

Fig. 2. Concentrations of noradrenaline (a), MHPG (b) and MHPG/NA ratio (c) in the paraventricular nucleus of naive and tolerant rats, 30 min after injection of saline i.p. or morphine (30 mg/kg i.p.). Each value is expressed as the per cent change from values for placebo-treated control rats within each treatment condition ($n = 5$–6 per group; means ± S.E.M.). $^{*}P < 0.05$; $^{**}P < 0.01$ vs. their respective controls; $^{+++}P < 0.001$ vs. naive animals injected with morphine.

3.2. Noradrenaline levels and turnover in the paraventricular nucleus after acute or chronic morphine administration

Acute administration of morphine (30 mg/kg i.p.) to naive rats did not alter the noradrenaline concentration in the paraventricular nucleus (Fig. 2a; control level, 3392 ± 274.4 ng/g). However, this dose of morphine significantly increased the production of MHPG ($P < 0.01$; Fig. 2b; control level, 406.2 ± 81.6 ng/g), as well as noradrenaline turnover (as estimated by the MHPG/NA ratio; $P < 0.05$;

Fig. 2c; control level, 0.13 ± 0.03). These changes were seen at the time of corticosterone secretion after the acute injection of the opioid. Fig. 2 also depicts noradrenaline content and turnover and MHPG production in the paraventricular nucleus of rats rendered tolerant to morphine. The morphine-pelleted group injected acutely with saline on day 7 showed no changes in MHPG concentration (Fig. 2b) or noradrenaline turnover (Fig. 2c) when compared with the corresponding naive group, indicating that tolerance develops towards the noradrenaline-turnover accelerating effect of morphine. There were no significant differences in MHPG production or in noradrenaline turnover between the two morphine-pelleted groups injected with saline or morphine i.p. Nevertheless, the tolerant group injected with morphine showed a significantly lower MHPG production ($P < 0.001$) and noradrenaline turnover ($P < 0.001$) than the naive rats given morphine. The correlation coefficient between MHPG/NA ratio and corticosterone concentration was 0.516 ($P < 0.05$), indicating that these two variables are correlated.

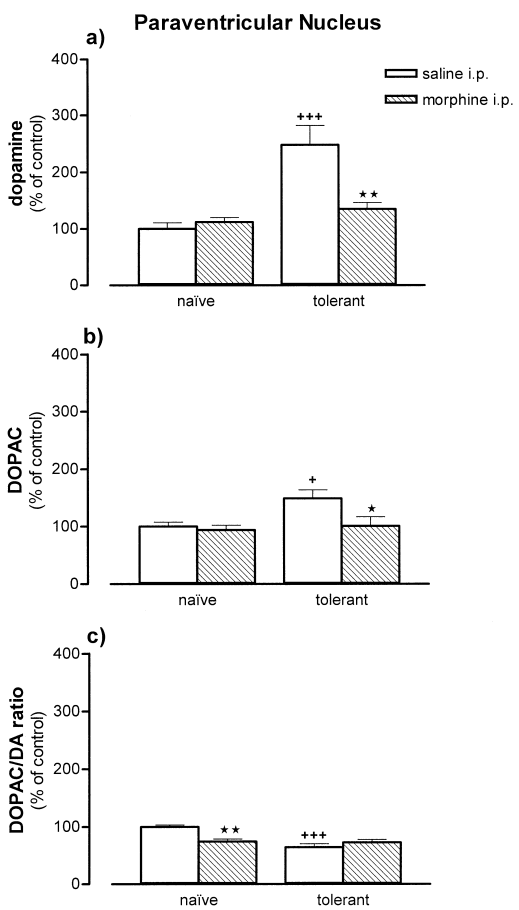

Fig. 3. Concentrations of dopamine (a), DOPAC (b) and DOPAC/DA ratio (c) in the paraventricular nucleus of naive and tolerant rats, 30 min after injection of saline i.p. or morphine (30 mg/kg i.p.). Each value is expressed as the per cent change from values for placebo-treated control rats within each treatment condition ($n = 4$–7 per group; means ± S.E.M.). $^{*}P < 0.05$; $^{**}P < 0.01$ vs. their respective control groups injected with saline i.p.; $^{+}P < 0.05$; $^{+++}P < 0.001$ vs. naive animals injected with saline i.p.

3.3. Dopamine concentration and turnover in the paraventricular nucleus after acute and chronic morphine administration

Acute administration of morphine (30 mg/kg i.p.) to naive rats did not modify significantly either the concentration of dopamine (Fig. 3a; control level, 556 ± 59.5 ng/g) or the production of DOPAC (Fig. 3b; control level, 513 ± 39.5 ng/g). However, the turnover of dopamine (as estimated by the DOPAC/dopamine–DA-ratio) decreased significantly ($P < 0.01$; Fig. 3c; control level, 0.93 ± 0.03). In rats rendered tolerant to the opioid, the content of dopamine and DOPAC production increased significantly ($P < 0.001$, $P < 0.05$, respectively) 30 min after saline injection (Fig. 3a,b), whereas dopamine turnover was lower ($P < 0.001$) than that in the control group also receiving saline (Fig. 3c). However, acute morphine injection to the tolerant group did not modify the dopamine concentration, DOPAC production or dopamine turnover when compared with the naive group also receiving morphine acutely.

3.4. cAMP levels in the paraventricular nucleus after acute or chronic morphine administration

The concentration of cAMP was measured in the hypothalamic paraventricular nucleus obtained from rats implanted with placebo or morphine pellets. Morphine (30 mg/kg i.p.) administration to placebo treated rats had no effects on cAMP levels (3.14 ± 0.16 nmol/g) when compared with placebo control rats injected with saline (3.0 ± 0.19 nmol/g). In morphine-tolerant rats, cAMP levels 30 min after saline (i.p.) injection (3.3 ± 0.14 nmol/g) were similar to those observed in placebo-treated animals. Similarly, the cAMP concentration remained unchanged after administration of morphine (30 mg/kg i.p.) to rats chronically treated with the opioid (3.03 ± 0.14 nmol/g).

4. Discussion

The mechanisms underlying the effects of chronic opioid administration and the manner in which responses to morphine are organised in the different brain nuclei are not well known. In this study, we investigated the adaptive changes in noradrenergic and dopaminergic neurons projecting to the paraventricular nucleus, and the concomitant opioid modulation of cAMP formation upon exposure to the preferential μ-opioid receptor agonist, morphine. Concomitantly, we studied the corticosterone response as an index of hypothalamus–pituitary–adrenocortical axis activity.

Consistent with the well-known stimulatory effect of opioids on hypothalamus–pituitary–adrenocortical activity

(Martínez et al., 1990), the present results show that the acute injection of morphine into placebo-pelleted rats produces an increase in the release of corticosterone. The possible correlation between this alteration and the changes in the activity of neuronal systems such as noradrenergic and dopaminergic neurons has not been established yet. The present data indicate that acute stimulation of opioid receptors leads to overproduction of the brain noradrenaline metabolite MHPG and elevation of the MHPG/NA ratio (an index of noradrenaline turnover in the paraventricular nucleus; Lookingland et al., 1991), indicating an augmented noradrenaline turnover. By contrast, the DOPAC/DA ratio (which reflects the activity of dopamine neurons; Manzanares et al., 1990) was reduced, suggesting that morphine decreased the activity of dopamine neurons projecting to the paraventricular nucleus. The alterations in catecholamine turnover were seen at the time of corticosterone secretion. These findings strongly suggest that noradrenergic terminals innervating the paraventricular nucleus have a stimulatory effect on hypothalamus–pituitary–adrenocortical axis activity. The results presumably reflect an indirect action of morphine on the axis through noradrenergic neurons, together with the involvement of noradrenaline in the morphine-induced neuroendocrine response.

μ-Opioid receptor mRNA has been detected in the hypothalamic paraventricular nucleus, and it has been suggested that μ-receptors could be involved in the neuroendocrine regulation of the hypothalamus–pituitary–adrenocortical axis (Mansour et al., 1995). However, hypothalamic CRF neurons are innervated by noradrenergic afferents from other brain regions, mainly the A_1 and A_2 neuronal complexes and, to a lesser extent (8% at most), the A_6 group (Cunningham and Sawchenko, 1988). It has been demonstrated that the paraventricular nucleus contains α- and β-adrenoceptors and that noradrenaline interacts with the parvocellular division of the paraventricular nucleus (Owens and Nemeroff, 1991). Although the role of noradrenergic systems, in the secretion of CRF, is controversial (Plotsky et al., 1989), previous studies have shown that noradrenaline stimulates CRF gene expression in the paraventricular nucleus (Itoi et al., 1994) as well as CRF and vasopressin secretion (Widmaier et al., 1989), both of which trigger ACTH secretion and corticosterone release (Itoi et al., 1994). This response is decreased in rats immunoneutralised with an anti-CRF serum (Szafarczyk et al., 1987). In agreement with the present data, our previous studies with the whole hypothalamus have revealed an increase in noradrenaline turnover of rats acutely injected with morphine (Milanés et al., 1997). In addition, previous results have shown that administration of reserpine or noradrenergic receptor blockers antagonises morphine-induced corticosterone secretion, supporting the hypothesis that noradrenaline is a likely candidate to mediate the effects of opioids on the hypothalamus–pituitary–adrenocortical axis (Martínez-Piñero et al., 1994). Taken together, these results suggest that the ability of morphine to increase hypothalamus–pituitary–adrenocortical axis activity is secondary to the action of the drug to stimulate the activity of noradrenergic neurons.

The present data shows that after acute morphine injection in control rats, the turnover of dopamine in the paraventricular nucleus is reduced. The distribution of dopaminergic inputs through the paraventricular nucleus is not well known, although immunocytochemical studies have described fibres and terminals in the parvocellular part of this nucleus (Petrusz and Merchenthaler, 1992). Furthermore, it has been shown that dopaminergic neurons in the paraventricular nucleus have a role in the regulation of the hypothalamus–pituitary–adrenocortical axis (Borowsky and Kuhn, 1993). In fact, after acute exposure to cocaine or to its active metabolite cocaethylene, plasma ACTH and corticosterone concentrations increase, suggesting that dopamine can contribute to the regulation of the axis (Torres et al., 1996).

The present study indicates that chronic morphine exposure evokes a decrease in corticosterone secretion in parallel with a decrease in both the production of MHPG and noradrenaline turnover, which indicates that tolerance develops towards the noradrenergic activity accelerating effects of morphine in the paraventricular nucleus. This effect was observed in both tolerant groups treated acutely with saline or morphine. Since changes in noradrenaline turnover were observed concomitantly with changes in corticosterone secretion, the present results also suggest that the chronic actions of opioids on the hypothalamus–pituitary–adrenocortical axis might be mediated through noradrenergic systems projecting to the paraventricular nucleus, as indicated in previous studies of the hypothalamus (Martínez-Piñero et al., 1994). The present findings show that the dopamine turnover in the paraventricular nucleus after chronic morphine administration was similar to that found after acute injection of the opioid, indicating that tolerance did not develop towards the dopamine release-reducing effect of morphine. Therefore, it seems that noradrenergic neurons are more sensitive to the tolerance-producing effects of morphine than dopaminergic neurons are.

Major intracellular signalling systems, including the cAMP-dependent protein kinase A and the diacylglycerol-dependent protein kinase C pathways, have been implicated in the regulation of CRF synthesis and/or secretion (Emanuel et al., 1990). Recent results suggest that the protein kinase A pathway plays an essential role in CRF gene transcription under physiological conditions (Itoi et al., 1996). In the present study, we observed that the cAMP levels in the paraventricular nucleus did not change after acute or chronic morphine administration. The present results are in agreement with those of previous biochemical studies reporting that adenylate cyclase activity and cAMP are selectively modified after chronic morphine treatment in the nucleus accumbens, amygdala and locus

coeruleus, but not in several other brain regions (Valverde et al., 1997).

Previous studies have reported the involvement of α_1-adrenoreceptors of the paraventricular nucleus in stimulation of the hypothalamus–pituitary–adrenocortical axis, whereas the involvement of β-adrenoceptors may be minimal (Itoi et al., 1994). Thus, prazosin, an α_1-adrenoceptor antagonist, abolishes the corticosterone and ACTH response to noradrenaline and morphine (Itoi et al., 1994; Martínez-Piñero et al., 1994). The α_1-adrenoceptor subtype is coupled to GTP-binding protein, which leads to activation of protein kinase C, whereas the β-adrenoceptor subtype is coupled to adenylate cyclase. Since no changes in cAMP levels were seen in the paraventricular nucleus of rats receiving acute or chronic morphine, it is possible that the opioid regulates the activity of CRF neurons through noradrenaline release acting on α_1-adrenoceptors, although further studies are required to identify the mechanisms implicated.

In summary, our results show that acute morphine administration produces enhanced noradrenaline turnover in the hypothalamic paraventricular nucleus concomitantly with an increase in corticosterone secretion. It is, therefore, possible that noradrenaline released in this nucleus from fibres originating in brainstem cell groups may modulate CRF function in response to opioids. Further, the present study clearly demonstrates a compensatory change, in the opposite direction to that of the acute actions of the opioid, that occurs in noradrenergic neurons as a result of chronic morphine treatment: a decrease in noradrenaline turnover. Adaptive changes produced by morphine in the noradrenergic system innervating the paraventricular nucleus could be linked to the alterations in hypothalamus–pituitary–adrenocortical axis activity seen during morphine tolerance. Finally, the significance of changes in dopamine turnover after morphine administration and their relation with the axis clearly requires further study.

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