Alterations in Corticotropin-Releasing Factor and Vasopressin Content in Rat Brain during Morphine Withdrawal: Correlation with Hypothalamic Noradrenergic Activity and Pituitary-Adrenal Response

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ABSTRACT

The modification in the activity of noradrenergic neurons projecting to the hypothalamus and the pituitary-adrenal response during morphine withdrawal as well its correlation with alterations in corticotropin-releasing factor (CRF) and vasopressin (AVP) content in different brain areas was analyzed. Male rats were implanted with placebo (naïve) or morphine (tolerant/dependent) pellets for 7 days. On day 8, groups of rats received an acute injection of saline s.c. (control) or naloxone (1 mg/kg s.c.) and were decapitated 30 min later. After administration of naloxone to tolerant rats (withdrawal) we found a striking parallelism between an enhanced activity of hypothalamic noradrenergic neurons and an increased corticosterone secretion; concomitantly, the CRF but not the AVP content in the paraventricular nucleus was decreased, which might reflect an increased release of the peptide. During withdrawal, CRF content also was decreased in the arcuate nucleus, whereas no changes were found in the median eminence, dorsomedial, ventromedial nuclei or in the bed nucleus of the stria terminalis. AVP content levels were not modified in arcuate nucleus, supraoptic or in the suprachiasmatic nuclei. Present data suggest that a hypothalamic noradrenergic hypersecretion may be involved in a selectively increased activity of CRF neurons in the paraventricular nucleus and arcuate nucleus and then in the enhanced release of corticosterone induced by morphine withdrawal. However, we did not find any correlation between opioid withdrawal-induced alterations in the pituitary-adrenal axis and AVP modifications.

Although endogenous opioid peptides are thought to be involved in modulating the HPA axis, the effects of opioids on the axis have been a matter of controversy (Pechnick, 1993). In rats, acute administration of morphine and related opioid agonists produces an increased HPA activity (Ignar and Kuhn, 1990; Martínez et al., 1990; Alcaraz et al., 1993), which may suggest an involvement of CRF in opioid-induced neuroendocrine responses (Wang et al., 1996). Chronic exposure to these agonist results in the development of tolerance to and dependence on opioid-induced endocrine secretion (Martínez et al., 1990; González et al., 1991; Vargas et al., 1997).

Opioid withdrawal produces complex behavioral, autonomic and endocrine alterations in rats (Maldonado et al., 1992), including an activation of the HPA axis activity (Martínez et al., 1990; González et al., 1994; Vargas et al., 1997), possibly because of the overproduction of CRF. Although implication of noradrenergic neurotransmission in the locus ceruleus has been suggested for explaining morphine withdrawal (Nestler, 1992), and increased activity of noradrenergic cells of the locus ceruleus correlates with withdrawal behavior, the exact neurobiological mechanisms of opioid withdrawal have yet to be determined. There are a few reports concerning the characteristics of functional disturbances of hypothalamic noradrenergic neurotransmission during opioid tolerance/dependence. Previously, we have observed opposite effects of acute and chronic morphine exposure on hypothalamic NA content and turnover (Martínez- Piñero et al., 1994; Vargas et al., 1997), which suggests the involvement of hypothalamic noradrenergic terminals in the development of opioid tolerance/dependence.

Corticotropin-releasing factor produced in the parvicellular division of the PVN of the hypothalamus plays a major role in the regulation of HPA axis function, and its activity appears to be modulated by a complex interplay of endogenous opioid peptides (Maldonado et al., 1992). Recently, other peptides, such as the inhibitory peptide somatostatin, have been found to modulate CRF secretion (Martínez-Piñero et al., 1994). Since CRF is a well-known regulator of the HPA axis activity, any alteration in CRF distribution may help to explain the complex functional disturbances found in opioid withdrawal. In this respect, the presence of opioid receptors in different brain areas involved in HPA regulation suggests the involvement of these receptors in the control of CRF secretion (Martínez-Piñero et al., 1994; Vargas et al., 1997).

Nevertheless, the precise mechanisms underlying the changes in CRF content during opioid withdrawal have yet to be determined. In this study, we have analyzed the distribution of CRF and AVP in different brain areas of rats receiving chronic morphine administration and then subjected to withdrawal. We found a selective decrease in CRF content in the paraventricular and arcuate nuclei of tolerant rats, whereas no changes were found in other areas of the brain, such as the median eminence, dorsomedial, ventromedial nuclei or in the bed nucleus of the stria terminalis. AVP content levels were not modified in arcuate nucleus, supraoptic or in the suprachiasmatic nuclei. These findings suggest that a hypothalamic noradrenergic hypersecretion may be involved in a selectively increased activity of CRF neurons in the paraventricular nucleus and arcuate nucleus and then in the enhanced release of corticosterone induced by morphine withdrawal. However, we did not find any correlation between opioid withdrawal-induced alterations in the pituitary-adrenal axis and AVP modifications.

ABBREVIATIONS: HPA, hypothalamus-pituitary-adrenal; CRF, corticotropin-releasing factor; AVP, vasopressin; NA, noradrenaline; PVN, paraventricular nucleus; ME, median eminence; BNST, bed nucleus of the stria terminalis; DMN, dorsomedial nucleus; VMN, ventromedial nucleus; AN, arcuate nucleus; SON, supraoptic nucleus; SCN, suprachiasmatic nucleus; MHPG, 3-methoxy-4-hydroxyphenylethylene glycol; ACTH, corticotropin; CNS, central nervous system; RIA, radioimmunoassay; HPLC, high-performance liquid chromatography.
role in the regulation of ACTH release from anterior pituitary (Vale et al., 1981). Parvocellular cells, under a variety of circumstances, also synthesize AVP, which also has corticotrophic releasing activity (Franci et al., 1993). Considerable evidence suggests that, in addition to its role in ACTH secretion, CRF plays a key role in mediating behavioral, autonomic and endocrine responses to stress, thus raising the possibility that the central CRF system may act to integrate multimodal components of the organic response to different types of stress. On the other hand, there is pharmacological evidence that NA modulates the synthesis and release of hypothalamic CRF (Widmaier et al., 1989; Itoi et al., 1994), and it has been proposed that opioids can affect HPA axis activity indirectly by altering noradrenergic neurotransmission (Suemaru et al., 1989; Martínez-Piñeiro et al., 1994).

Because the hypothalamus is a key modulator and integrator of numerous behavioral and physiological functions, it is important to determine the possible changes in CRF/AVP within different hypothalamic nuclei during opioid withdrawal. To elucidate the link between CRF, AVP and hypothalamic NA turnover during opioid withdrawal, we examined the changes of CRF/AVP levels and noradrenergic activity in the hypothalamus of rats made dependent on morphine and after naloxone-precipitated withdrawal. For that, CRF and AVP content were measured in different nuclei and regions (including the PVN, median eminence, ventromedial, dorsomedial, supraoptic, suprachiasmatic, arcuate nuclei and the bed nucleus of the stria terminalis). These structures were chosen on the basis of an attempt to establish the areas related to stress response that are modified during opioid withdrawal, which might aid in defining neural circuitry mediating long-term adaptation to opioids. Concomitantly, in parallel experiments, the hypothalamic NA and MHPG (its cerebral metabolite) levels and plasma corticosterone concentration (as a marker of HPA axis activity; Yokoe et al., 1988) were evaluated.

Methods

Animals. Male Sprague-Dawley rats (200–210 g at the beginning of treatment) were housed four to five per cage under a 12-h light/dark cycle in a room with controlled temperature (22 ± 1°C), humidity (50 ± 10%) and food and water available ad libitum.

Experimental procedure. On the basis of previous studies (González et al., 1994; Vargas et al., 1997), rats were rendered tolerant/dependent on 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 anesthesia. This treatment results in a profound state of tolerance and dependence. Control animals were implanted with placebo pellets containing lactose at the same time schedule. On day 7 animals were injected with saline s.c. or naloxone HCl (1 mg/kg s.c.) and were observed for opioid withdrawal behavior (wet-dog shakes, teeth chattering, salivation, lacrimation, locomotion, rhinorhoea and ptosis) for 30 min. At the end of this period animals were sacrificed, and analytical studies were conducted. There were four experimental groups: 1) chronic placebo (naïve)-acute saline s.c.; 2) chronic placebo-acute naloxone s.c.; 3) chronic morphine-acute saline s.c.; 4) chronic morphine-acute naloxone s.c.

Corticosterone assays. At the end of the treatment, rats were sacrificed by decapitation between 10:00 and 11:00 a.m. to avoid circadian variations in plasma levels of corticosterone or in the hypothalamic content and turnover of NA. Trunk blood was collected into ice-cooled tubes containing 5% ethylenediaminetetraacetic acid and then was centrifuged (2500 rpm; 4°C; 15 min). Plasma was separated and stored at −30°C until assayed for corticosterone. Plasma levels of corticosterone were estimated with a commercially available kit for rats (125I-corticosterone radioimmunoassay, ICN Biomedicals, Costa Mesa, CA). The sensitivity of the assay was 0.40 ng/ml. The 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.

Estimation of hypothalamic NA and MHPG. After decapitation, the brains were removed, fresh-frozen and stored at −80°C until use. For estimation of NA and MHPG, the hypothalamic tissue (delinated by the optic chiasma rostrally, the anterior margin of the mamillary bodies caudally and the lateral hypothalamic sulci) was excised. Dorsally, the cut was performed at about 3 mm from the ventral surface. NA and its metabolite in the CNS (MHPG) were determined by HPLC with electrochemical detection. Each tissue was weighed, placed in a dry-cooled polypropylene vial and homogenized with a Polytron-type homogenizer (setting 5 for 30 s) in 1 ml perchloric acid (0.1 M). The homogenates were then centrifuged (15,000 rpm; 4°C; 15 min) and the supernatants taken for analysis. Two aliquots of the supernatant from the same tissue sample were used, one for analysis of NA and the other for analysis of MHPG. The aliquot for NA analysis was filtered through 0.22 μm GV (Millipore, Bedford, MA) and 10 μl of each sample was injected into a 5-μm C18 reverse-phase column (Waters, Milford, MA). 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 95.5:4.5 (v/v) mixture of water and methanol with sodium acetate (50 mM), citric acid (20 mM), 1-octyl-sodium sulfate (3.75 mM), di-n-butylamine (1 mM) and ethylenediaminetetraacetic acid (0.135 mM), adjusted to pH 4.3. The flow rate was 0.9 ml/min and chromatographic data were analyzed with a Millenium 2010 Chromatography Manager (Millipore) equipment. Because most of MHPG is present in a sulfate conjugate form in the rat CNS, the method for the determination of total MHPG in the hypothalamus is based on the acid-catalyzed hydrolysis of MHPG-sulfate (Artigas et al., 1986; Lookingland et al., 1991). The aliquots for MHPG analysis were kept in polypropylene, screw-capped tubes for 5 min in a water bath at 100°C. The tubes were then cooled on ice and centrifuged (4000 rpm; 4°C; 10 min). The supernatant was filtered through a 0.22 GV and hydrolyzed samples were injected (50 μl) into the HPLC equipment. The eluent for MHPG determination was as described above, but without 1-octyl-sodium sulfate. Under these conditions, MHPG eluted at 4.80 to 5 min. NA and MHPG were quantified by reference to calibration curves run at the beginning and the end of each series of assays. The hypothalamic content of NA and MHPG was expressed as nanograms per gram wet weight of tissue.

CRF and AVP assays. After decapitation the brains were removed quickly, frozen on dry ice and stored at −80°C. Serial sections of 300 μm were cut, and different anatomical structures were micropunched: PVN, VMN, DMN, AN, SON, SCN, BNST and the ME. Bilateral tissue samples pooled from two rats were placed in 100 μl of cold 0.2 N HCl in microfuge tubes (500 μl capacity) and were stored at −70°C until assayed.

Hypothalamic peptide content was measured with the specific RIA developed in the laboratory of cellular biology of INSERM (Nancy, France). Tissues were homogenized by ultrasonic disruption. Protein concentrations were determined on an aliquot (100 μl) of homogenate by the method of Lowry et al. (1951). The rest of the homogenate was centrifuged at 4,000 rpm for 20 min at 4°C. The acid extracts were shared into two aliquots, stored at −40°C and the lyophilized just before the RIA. The amounts of CRF in the tissue extracts were determined with an antibody raised in rabbit against rat CRF (Neo-system, Strasbourg, France). It reacted with human or rat (Tyr)-CRF but did not show cross-reactivity (<0.0001%) with AVP, oxytocin, thyrotropin-releasing or luteinizing hormone-releasing hormones or substance P. Just before RIA, the lyophilized samples were reconstructed in RIA buffer (0.05 M tris-HCl, 1% bovine serum albumin, 0.1%
Triton X-100, pH 7.5) and incubated at 4°C for 48 h with the antibody at a dilution of 1:10,000. Then the tracer (125I-Tyr-rat/human CRF, 2200 Ci/mm, NEN, Boston, MA) was added. Horse serum (100 µl) was added before the separation of bound peptide with charcoal-dextran mixture. After centrifugation (4000 rpm for 30 min at 4°C), the sample radioactivity was measured in the supernatant with a gamma counter coupled to a microcomputer (MDA 312 system, Kontron). The sensitivity of assay was 10 pg/tube. The intra- and interassay coefficients of variation were 6.5% and 9%, respectively.

The amounts of AVP in the tissue extracts were determined with an antibody raised against AVP (P1, dilution 1:25,000 final) in rabbit. The tracer was the 125I-labeled AVP (NEN, 2200 Ci/mmol). The antibody cross-reactivity with Arg-AVP and Lys-AVP was 100%, 1.25% with vasotocin, 0.07% with pressinoic acid and 0.002% with oxytocin. Assay sensitivity was 1 pg/tube; intra- and interassay variations were 1.4% and 12%, respectively.

Statistical analysis. The data are expressed as means ± S.E.M. Plasma levels of corticosterone and cerebral concentration of CRF, AVP, NA, MHPG and the MHPG/NA ratio were analyzed by a computer program with analysis of variance followed by the Newman-Keuls test. Significance level was taken as P < .05.

Drugs and chemicals. 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); NA bitartrate, MHPG hemipiperazinium salt (used as HPLC standards) and naloxone HCl, were purchased from Sigma Chemical Co. (St. Louis, MO). Naloxone HCl was prepared fresh every day, dissolved in sterile 0.9% NaCl (saline).

Results

Characteristic abnormal behavioral signs (escape behavior, wet-dog shakes, teeth chattering, locomotion, salivation, lacrimation, rhinorrhea and ptosis), showing the expression of withdrawal syndrome, were observed after s.c. injection of naloxone 3 days after the termination of the implantation of morphine pellets for 5 days.

Hypothalamic noradrenergic activity and corticosterone secretion. In control (naïve) rats, injection of naloxone (1 mg/kg s.c.) did not alter hypothalamic NA and its metabolite MHPG concentration (1203 ± 57 ng/g and 147.8 ± 13 ng/g vs. 1158 ± 83 ng/g and 109 ± 37 ng/g, respectively) or NA turnover (as measured by the MHPG/NA ratio; 0.10 ± 0.01 vs. 0.087 ± 0.01) (fig. 1, hatched vs. open columns). Concomitantly, plasma corticosterone levels were not modified 30 min after naloxone injection to naïve rats, as compared with the control group injected with saline (166 ± 13 ng/ml vs. 168 ± 8 ng/ml; fig. 2).

Figure 1 depicts hypothalamic NA content and turnover for rats rendered tolerant to and dependent on morphine. The morphine-pelleted group injected with saline had significantly (P < .001) higher levels of NA (2505 ± 203 ng/g) than the control placebo-pelleted group, whereas the MHPG content (27 ± 6 ng/g) and MHPG/NA ratio (0.011 ± .002) were significantly (P < .05) lower in tolerant than in naïve rats. Plasma corticosterone concentration did not show any significant change 30 min after saline administration to morphine-pelleted rats (142 ± 17 ng/ml) compared with the corresponding control group (fig. 2).

In rats withdrawn from repeated morphine treatment by naloxone injection, hypothalamic NA content decreased significantly (1192 ± 61 ng/g) significantly (P < .001), whereas the MHPG content (466 ± 103 ng/g) and the MHPG/NA ratio (0.20 ± .04) increased significantly (P < .001) compared with the tolerant group injected with saline (fig. 1). Furthermore, the corticosterone secretion increased (435 ± 27) significantly (P < .001) during withdrawal compared with the saline-injected group (fig. 2).

Effects of morphine tolerance and abstinence on CRF levels. Figure 3 depicts that in rats receiving placebo
Table 1 shows changes in CRF content in the DMN, VMN, AN and BNST after different treatments. In naive rats, acute administration of naloxone significantly decreased CRF levels in DMN (P < .05) and in VMN (P < .01), whereas it was increased (P < .05) in the AN. Significant differences were not found in the BNST between saline and naloxone administration to naive rats. In morphine-pelleted rats CRF content was decreased in the DMN (P < .05), VMN (P < .01) and AN (P < .01), as compared with naive groups injected with saline. No changes in the CRF content were observed after naloxone injection to the tolerant group in any of the nuclei studied.

Effects of morphine tolerance and abstinence on AVP levels. Figure 4 depicts AVP levels in the PVN and ME for placebo- or morphine-pelleted rats injected acutely with saline or naloxone. Acute injection of naloxone to naive rats did not modify the AVP content in the PVN (32 ± 3 ng/mg vs. 30 ± 2 ng/mg). Similarly, injection of naloxone to morphine-pelleted rats induced no changes in the AVP levels (21 ± 2 ng/mg vs. 28 ± 2 ng/mg). When the content of AVP was measured in the ME, acute administration of naloxone to naive or to tolerant rats produced no changes in the AVP content (naive: 326 ± 43 ng/mg vs. 445 ± 58 ng/mg; tolerant: 381 ± 49 ng/mg vs. 296 ± 19 ng/mg). Table 2 depicts AVP levels for the AN, SON and SCN. Administration of naloxone to naive rats did not modify significantly the AVP levels in any of those nuclei. In morphine-pelleted rats, a decrease in the AVP content in the AN and in the SON (P < .05) was observed. Naloxone had no effect on the AVP content in morphine-treated rats.

Discussion

The mechanisms underlying the effects of chronic opioid administration and the way that responses to morphine are organized in the brain are not well known. Given the substantial innervation of the PVN by endogenous opioid and noradrenergic inputs, and the proposal role for catecholamines in opiate abuse (Nestler, 1992; Self and Nestler, 1995), we have investigated the adaptive changes in noradrenergic neurons projecting to the hypothalamus and the concomitant modifications in the HPA axis activity as well as in CRF/AVP content in different hypothalamic nuclei in rats withdrawn from repeated morphine treatment. The morphine pellet implantation induced a physical dependence, as shown by naloxone-precipitated abstinence signs (body shakes, teeth chattering, lacrimation, ptosis and so on). Consistent with previous reports (Ignar and Kuhn, 1990; Martínez et al., 1990; González et al., 1994), we found that
chronic morphine treatment also produced neuroendocrine dependence, as shown by the increased corticosterone secretion 30 min after naloxone injection. At present, the possible correlation between these alterations and the changes in CRF and other ACTH secretagogues has not been well established, such as AVP, or in the activity of some neurotransmitters, such as NA, which has been implicated in the control of CRF/AVP neurons at hypothalamic levels.

The results of the present study show that morphine withdrawal produces a decrease in hypothalamic NA content, an overproduction of the NA metabolite MHPG and an elevation of CRF/AVP neurons at hypothalamic levels. These changes were observed at the time of reduced CRF content in the PVN and increased corticosterone secretion. Present data indicate clearly that dependence is associated with an increase in noradrenergic activity in the hypothalamus and that hypothalamic noradrenergic terminals could have a stimulatory effect on the HPA axis and strongly suggest a critical role of NA in opioid withdrawal-induced neuroendocrine response. Because changes in NA turnover were observed at the time of corticosterone secretion, the present results may indicate that the alteration of HPA axis activity during withdrawal could be mediated through noradrenergic pathways, as proposed recently (Vargas et al., 1997). The mechanisms by which opioid withdrawal produces activation of HPA axis are not well established. Simultaneous measurement of hypothalamic NA content and turnover and corticosterone secretion, as carried out in the present experiment, indicates that an increase in NA release occurs during withdrawal concomitantly with corticosterone hypersecretion, which emphasizes that the opioid withdrawal-stimulated HPA axis can involve the release of NA.

The opposite effect was observed during tolerance, because an increased hypothalamic NA content and a reduced MHPG/NA ratio upon chronic morphine exposure were found, which indicates that the turnover of hypothalamic NA decreases during chronic opioid administration. In addition, there was no change in corticosterone secretion. Because acute morphine administration to naive rats increases hypothalamic MHPG production, NA turnover and corticosterone secretion (Attila, 1989; González et al., 1994; M. V. Milanés and M. L. Laorden, unpublished observations), the present results indicate that tolerance develops to the NA turnover and release increasing effects of morphine in the hypothalamus, as well as toward the HPA axis activity-accelerating effect of the opiate.

Excitation of the HPA axis is driven by central stress circuits (Herman and Cullinan, 1997). Notable among these are the brainstem catecholamine-producing neurons, mainly the nucleus of the solitary tract and the locus ceruleus (Cunningham and Sawchenko, 1988), which project to the CRF-containing neurons of the PVN. Although there has been a controversy regarding the role of NA on CRF secretion (Plotsky et al., 1989), recent studies indicate that NA stimulates CRF gene expression in the PVN (Itoi et al., 1994) as well as CRF and AVP secretion (Raber et al., 1995; Widmaier et al., 1989), both triggering ACTH and corticosterone release (Itoi et al., 1994). The present data therefore further support the notion that hypothalamic noradrenergic terminals play a pivotal role in the neuroendocrine manifestation of opioid withdrawal.

CRF produced in the PVN plays a major role in the regulation of the HPA axis, which is co-regulated by AVP. Because the hypothalamus is a key modulator of numerous behavioral and physiological functions, it is important to determine the response of CRF and AVP neurons within hypothalamic nuclei after administration of opioids. In addition, increased activity of the brain CRF system is involved in the pathophysiology of anxiety and stress, which are some of the psychiatric consequences of chronic opioid abuse and withdrawal. In the present study we show that administration of naloxone to morphine-dependent rats produces different changes in the CRF and AVP levels in discrete hypothalamic nuclei as well as in the BNST. These nuclei contain immuno-reactive CRF or AVP, CRF/AVP cell bodies, fibers or receptors (Owens and Nemeroff, 1991) as well as opioid-receptor mRNA and binding (Mansour et al., 1995). A decrease of CRF content in the PVN was observed when nalox-
one was administered to tolerant rats. The decrease of CRF levels was correlated with the simultaneous increase that was observed in plasma corticosterone concentration as well as in noradrenergic release during morphine withdrawal. Although the measurement of peptide concentration alone cannot distinguish between synthesis, release or degradation, differences between treatment groups clearly represent alterations in the activity of neurons in function of the drug or the pretreatment (Owens and Nemeroff, 1991) and a decrease in the peptide levels in a certain brain area usually reflects an increased release and subsequent degradation of the neuropeptide (Sarnyai et al., 1992). So, withdrawal-induced reduction of CRF in the PVN probably was caused by the release of CRF into the hypothalamic-pituitary portal circulation then activating the pituitary-adrenal axis. However, we did not observe any changes in the CRF content in the ME during withdrawal.

The hypothalamic levels of CRF are decreased 15 min after restraint stress (Moldow et al., 1987). Both stress and opioid withdrawal activate the release of stress hormones ACTH and corticosterone in rats (Igner and Kuhn, 1990; González et al., 1994). Because opioid withdrawal affects the HPA axis in a way similar to that of stress, our results might indicate an increased depletion of CRF during withdrawal. Because peptides must be synthesized de novo, the synthesis of peptides must match their release over time. One thus can state that increased synthesis implies increased release of these peptides. Because opioid withdrawal increases the HPA activity, a presumed increase of neuronal CRF synthesis and release in the PVN could contribute to the endocrine effects of abstinence, although it would be necessary to determine the CRF mRNA expression. In a previous study, Lightman and Young (1988) showed a marked increase in parvocellular CRF mRNA after acute ether stress and after naloxone-induced morphine withdrawal. C-fos protein expression is regarded as a marker for neuronal activation and therefore can be used to map functional pathways in the CNS (Cullinan et al., 1996), and naloxone-precipitated morphine withdrawal induces expression of c-fos protein mRNA in the PVN, which could be mediated through the augmented release of NA into the PVN (Harbuz et al., 1991). However, studies showing transcriptional activation of CRF and AVP genes in the PVN 5 min after acute stress have failed to detect concomitant up-regulation of CRF mRNA (Kovács and Sawchenko, 1996).

The coexistence of CRF and AVP in the same parvocellular subdivision of the paraventricular neurons has been established (Piecut and Joseph, 1986; Whitnall, 1988). In addition, it is known that catecholaminergic innervation of the PVN stimulates the synthesis and the release of AVP (Alonso et al., 1986). However, although it is well established that the HPA axis is co-regulated by AVP, the role of this peptide in the HPA adaptation during opioid tolerance/dependence has not been investigated. Present results showing an absence of changes of AVP in the PVN and ME during morphine withdrawal may suggest that AVP in these regions could not participate in the pituitary-adrenal adaptation during chronic opioid exposure. Another possibility is that activation of AVP might follow a time course distinct from CRF, as has been observed in a previous work (Kovács and Sawchenko, 1996).

At present, it is still unclear as to which area of the brain is involved mainly in the expression of opioid tolerance/dependence signs. The regulation of CRF and AVP in other nuclei different from the PVN has not been investigated thoroughly. Present data reflect how other hypothalamic and extrahypothalamic neurons containing CRF also are modified after acute or chronic morphine treatment. The results reported here provide evidence that adaptation to repeated opioid exposure or during withdrawal occurs as a change in CRF and/or AVP response in defined brain areas. Our data demonstrate that, depending on the nucleus, a differential response of CRF and AVP neurons to chronic morphine or to naloxone administration can be observed. Acute administration of naloxone to naïve rats was found to result in decreased levels of CRF in the DMN and in the VMN, whereas an increase of the peptide was observed in the AN, without changes in the BNST. After chronic morphine administration, the VMN and the DMN showed decreased CRF, and only AN shows a significant alteration (a decrease) during withdrawal. As for AVP, the AN and the SON showed a reduction in the content of this peptide in morphine-pelleted rats, whereas no changes were observed in these nuclei during withdrawal. We failed to observe modifications of AVP content in the SCN after chronic morphine administration or in morphine-withdrawn rats.

The presence of nerve terminals containing endogenous opioid peptides impinging on CRF neurons (Roth et al., 1989) as well as opioid receptors (Mansour et al., 1995) has been demonstrated. Because of the vast variety of functions over which CRF may exert a modulatory influence as neurotransmitter, and because this peptide integrates the physiological and behavioral responses of the organism to different stressors, our data might suggest that the response of CRF neurons to opioid administration may contribute to the physiological, behavioral and emotional alterations that occur in opioid-dependent subjects. Concerning our data, a coordinated measurement of CRF/AVP mRNA, (experiments in progress) will help to verify the exact nature of the changes found after morphine administration and during opioid withdrawal. In summary, the present results are consistent with a stimulatory effect of opioid withdrawal on the activity of noradrenergic neurons in the hypothalamus, which in turn might activate CRF release from the PVN then ACTH and corticosterone secretion. In addition, the CRF and AVP changes in other areas indicate a complex response to naloxone, chronic morphine and opioid withdrawal.

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