Changes in Catecholaminergic Pathways Innervating Paraventricular Nucleus and Pituitary-Adrenal Axis Response during Morphine Dependence: Implication of $\alpha_1$- and $\alpha_2$-Adrenoceptors

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ABSTRACT

We have previously shown an enhanced activity of the pituitary-adrenal response in rats dependent on morphine, which occurs concomitantly with an increase in the activity of catecholaminergic terminals in the hypothalamic paraventricular nucleus (PVN). The present study examined the possible role of noradrenergic system in the regulation of opioid withdrawal-induced activation of the hypothalamus-pituitary-adrenocortical (HPA) axis activity. Rats were given morphine by s.c. implantation of morphine pellets for 7 days. On the seventh day, morphine withdrawal was induced by s.c. administration of naloxone (1 mg/kg), rats were sacrificed 30 min later, and changes in noradrenaline (NA) turnover (estimated by the 3-methoxy-4-hydroxyphenylethylene glycol/NA ratio) and in dopamine turnover (estimated by the 3,4-dihydroxyphenylacetic acid/dopamine ratio) in the PVN (HPLC with electrochemical detection) and in plasma corticosterone levels were determined. We found a parallelism between the behavioral signs of withdrawal, an increased activity of noradrenergic and dopaminergic terminals in the PVN, and the hypersecretion of the HPA axis. Pretreatment with $\alpha_1$- or $\alpha_2$-adrenoceptor antagonists prazosin or yohimbine, respectively, 15 min before naloxone administration significantly prevented the withdrawal-induced corticosterone hypersecretion and attenuated the behavioral signs of morphine withdrawal. In addition, biochemical analysis indicated that both prazosin and yohimbine completely abolished the withdrawal-induced increase in NA turnover in the PVN. In contrast, neither prazosin nor yohimbine modified the hyperactivity of dopaminergic terminals in the PVN during withdrawal. Collectively, these data suggest that the secretory activity in the HPA axis after morphine withdrawal results from an increase in noradrenergic activity that is dependent on $\alpha_1$- and $\alpha_2$-adrenoceptor activation. Activation of dopaminergic pathways might not contribute to the neuroendocrine response during withdrawal.

The repeated use of opiates induces adaptive changes in the central nervous system leading to the development of tolerance and dependence. There is considerable evidence implicating the noradrenaline (NA) system in the locus ceruleus as a mediator of many of the physical withdrawal behavior characteristics of opioid dependence (Nestler et al., 1993). Additionally, different studies have established the mesolimbic dopamine (DA) system as a major neural substrate of the reinforcement produced by opioids (Self and Nestler, 1995). Many compensatory mechanisms have been observed in the locus ceruleus as well as in the ventral tegmental area and nucleus accumbens in association with chronic exposure to morphine, including alteration in gene expression (Nestler et al., 1993).

In rats dependent on morphine, the hypothalamus-pituitary-adrenal (HPA) axis is characterized by a marked response after naloxone-induced withdrawal (Martínez et al., 1990; Pechnick, 1993). In particular, there is an increase in the release of adrenocorticotropic hormone (ACTH) and corticosterone (Pechnick, 1993; Vargas et al., 1997). Furthermore, increased activity of the HPA axis can be seen at hypothalamic level, where an induction of corticotropin-releasing factor (CRF) messenger RNA transduction occurs during morphine withdrawal (Lightman and Young, 1988). This alteration in the responsiveness of the HPA axis appears not to be due to a direct effect of opioids on CRF release and probably involves pathways impinging on the paraven-

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ABBREVIATIONS: NA, noradrenaline; DA, dopamine; HPA, hypothalamus-pituitary-adrenocortical; ACTH, adrenocorticotropic hormone; CRF, corticotropin-releasing factor; PVN, paraventricular nucleus; MHPG, 3-methoxy-4-hydroxyphenylethyl alcohol; DOPAC, 3,4-dihydroxyphenylacetic acid.
tricular nucleus (PVN) (Vargas et al., 1997; Milanes et al., 1998).

The CRF-containing neurons of the PVN have been shown to receive noradrenergic inputs, which travel via the ventral noradrenergic bundle from catecholaminergic cell groups of the brainstem (Cunningham and Sawchenko, 1988). These noradrenergic inputs appear to participate in the activation of the PVN because their electrical stimulation induces CRF secretion that can be blocked by $\alpha_1$-antagonists (Plotzky, 1987). Furthermore, i.c.v. injection of noradrenaline or $\alpha_1$-agonists causes increases in the secretion of CRF and ACTH and induces CRF mRNA expression (Lookingland et al., 1991; Gunion et al., 1992; Ito et al., 1994). An increase in the NA and DA turnover in the PVN has been shown to occur in rats withdrawn from morphine, in parallel to the activation of the HPA axis (Vargas et al., 1997; Milanes et al., 1998). These changes in catecholaminergic turnover were accompanied by a decrease in the CRF content in the PVN, suggesting an increased release of CRF during withdrawal (Milanes et al., 1998).

The following studies examined the possibility that the enhanced response of the HPA axis during naloxone-induced withdrawal arises from the activation of $\alpha_1$- and/or $\alpha_2$-adrenoceptor. This hypothesis is suggested by the observations that the increase in corticosterone secretion after acute injection of morphine to naive rats was partially antagonized by administration of $\alpha_1$-adrenoceptor antagonists (Martinez-Pinero et al., 1994). In the current studies, injections of prazosin and yohimbine were performed in naive and morphine-dependent rats before naloxone administration, and the HPA function was measured by the changes in plasma corticosterone. In addition, the content of NA, DA and their metabolites in the PVN were measured to investigate whether the changes in catecholaminergic turnover during dependence are modified by $\alpha$-adrenoceptor manipulations.

**Materials and Methods**

**Animals and Drug Treatments.** 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. The animals were cared for in accordance with local committee and the National Institutes of Health ethical guidelines. Because stress can affect the activity of the HPA axis, the experimental design included efforts to reduce the potential effects of stress. Animals were handled daily (between 9:00 AM and 10:00 AM) for 7 days before the experimental day in the experimental room to adapt them to manipulation and minimize nonspecific stress responses. On the basis of previous studies (González et al., 1994; Vargas et al., 1997), rats were rendered tolerant to and 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 pelletting method provides continuous exposure to morphine and has been shown to induce both tolerance and dependence as measured behaviorally and biochemically (Couceyro and Douglass, 1995; Vargas et al., 1997). Control animals were implanted with placebo pellets containing lactose on the same time schedule. On day 7, animals were injected i.p. with vehicle, prazosin (1 mg/kg), or yohimbine (2 mg/kg) and 15 min later received saline (s.c.) or naloxone (1 mg/kg s.c.) and then were observed for behavioral signs of withdrawal. The incidence of teeth chattering, piloerection, lacrimation, rhinorrhea, spontaneous jumping, tremor, and ptosis were scored for 30 min. These behavioral signs are reliable markers of opioid withdrawal in morphine-dependent rats and have previously been used as indices of the degree of dependence (Maldonado et al., 1992). At the end of this period, animals were sacrificed and analytical studies were conducted. The 12 experimental conditions were as follows: placebo plus vehicle or prazosin or yohimbine plus saline (control); placebo plus vehicle or prazosin or yohimbine plus naloxone (naloxone control); morphine plus vehicle or prazosin or yohimbine plus saline (chronic morphine treatment); and morphine plus vehicle or prazosin or yohimbine plus naloxone (naloxone-precipitated withdrawal).

**Rats’ weight gain** was checked during treatment to ensure that morphine was liberated correctly from the pellets because it is known that chronic morphine treatment induces a decrease in body weight gain due to a lower caloric intake (Berhow et al., 1995). In addition, the day of experiment weight loss was determined as the difference between the weight determined immediately before saline or naloxone injection and a second determination made 30 min later, immediately before killing.

**Corticosterone Assays.** At the end of the treatment, rats were sacrificed by decapitation between 10:00 AM and 11:00 AM to avoid circadian variations in plasma levels of corticosterone or in the hypothalamic content and turnover of NA and DA. Trunk blood was collected into ice-cooled tubes containing 5% EDTA and then was centrifuged (2500 rpm; 4°C; 15 min). Plasma was separated, divided into two aliquots, and stored at −80°C until assayed for corticosterone. Plasma levels of corticosterone were estimated, as a sensitive marker of the HPA axis activity, with a commercially available kit for rats (S1 corticosterone radioimmunoassay; ICN Pharmaceuticals, 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 Catecholamines and Their Metabolites in PVN.** After decapitation, the brains were removed rapidly, fresh-frozen, and stored immediately at −80°C until use. The hypothalamic tissue containing the PVN was dissected from a coronal brain slice according to the technique of Palkovits (1973) and the PVN corresponds to those in plates 25 and 26, 1800 to 2100 µm caudal to the bregma (Palkovits and Brownstein, 1988). NA, its metabolite in the central nervous system 3-methoxy-4-hydroxyphenylethylgycol (MHPG), DA, and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) were determined by HPLC with electrochemical detection. Bilateral tissue samples were weighed, placed in 600 ml of cold perchloric acid (0.1 M), and homogenized with a Polytron-type homogenizer (setting 4 for 30 s). The homogenates were then centrifuged (15,000 rpm; 4°C; 15 min) and the supernatants taken for analysis and filtered through 0.22-µm GF filters (Millipore, Bedford, MA). Two aliquots of the supernatant from the same tissue sample were used, the first for analysis of NA, DA, and DOPAC and the second for analysis of MHPG. Ten microliters of the first aliquot of each sample was injected into a 5-µm C18 reversed-phase column (Waters Associates, Millipore Corp., Milford, MA) 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 versus the Ag/AgCl reference electrode (Waters Associates). 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-octylsulfonate (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 analyzed with a Millennium 2010 chromatography manager (Millipore) equipment. DOPAC, NA, and DA 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. Because in the rat central nervous system most of MHPG is present in a sulfate conjugate form, the method for the determination of total MHPG in the PVN is based on the acid-catalyzed hydrolysis of MHPG-sulfate (Artigas et al., 2000...
taken as analytical grade. Prazosin and yohimbine were dissolved in sterile distilled water. MO). Naloxone HCl was dissolved in sterile 0.9% NaCl (saline) and DOPAC (used as HPLC standards), naloxone HCl, prazosin HCl, and Spain); NA bitartrate, MHPG hemipiperazinium salt, DA HCl, macy and Pharmaceutic Technology (School of Pharmacy, Granada, Spain); NA bitartrate, MHPG hemipiperazinium salt, DA HCl, were analyzed by two-way ANOVA followed by the Newman-Keuls test.

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, DA hemipiperazinium salt, DOPAC (used as HPLC standards), naloxone HCl, prazosin HCl, and yohimbine HCl were purchased from Sigma Chemical Co. (St. Louis, MO). Naloxone HCl was dissolved in sterile 0.9% NaCl (saline) and prazosin and yohimbine were dissolved in sterile distilled water. Drugs were prepared fresh every day. Other reagents were of analytical grade.

Data Analysis. The data are expressed as means ± S.E. Data from body weight loss, plasma corticosterone, and catecholamines were analyzed by two-way ANOVA followed by the Newman-Keuls test. One-way ANOVA followed by Dunnett’s multiple comparison test was used when required. Body weight gain in naive and in morphine-dependent rats was analyzed by unpaired Student’s t test. Behaviors were quantified as the number of animals exhibiting the sign/total number of animals observed, and data obtained were analyzed nonparametrically with the χ² test. Significance level was taken as P < .05.

Results

The weight of each animal was recorded on the days of pellet implantation and on the day of decapitation (day 7), before receiving any injection. In all experimental groups, rats treated chronically with morphine showed significantly (P < .001) lower body weight gain (21.66 ± 1.32 g) than animals receiving placebo pellets (52.46 ± 1.14 g). As shown in Table 1, the regimen of 7 days of morphine pellet implantation produced dependence, as shown by the ability of naloxone to precipitate standard signs of withdrawal. Significantly lower frequency or total suppression of four of the seven signs (piloerectio, lacrimation, rhinorrhea, and ptosis) was noted in the dependent group pretreated with prazosin before naloxone injection. Pretreatment with yohimbine before naloxone also produced a lower frequency of four of the seven signs (teeth-chattering, lacrimation, ptosis, and spontaneous jumping). Signs of withdrawal were not observed in the placebo groups receiving vehicle plus saline or vehicle plus naloxone. In addition, rats implanted with pellets of morphine receiving vehicle plus saline did not show any signs of abstinence.

Table 2 depicts that administration of naloxone (1 mg/kg) to control rats resulted in no significant changes in body weight loss when measured 30 min after drug injection. However, chronic morphine-treated animals showed an important weight loss (P < .001) 30 min after naloxone injection compared with the morphine-treated group injected with saline s.c. In morphine-dependent rats pretreated with prazosin or yohimbine there was also a significant weight loss 30 min after naloxone injection (P < .01 and P < .001, respectively) compared with the respective control groups receiving saline instead of naloxone. However, weight loss in the prazosin-pretreated group was significantly (P < .01) lower than that observed in the dependent group pretreated with vehicle (Dunnett’s test).

Effects of Adrenergic Antagonists on Withdrawal-Induced Corticosterone Secretion. Plasma corticosterone levels were not modified 30 min after naloxone injection to naive rats, but increased significantly during morphine withdrawal (Fig. 1A). In morphine-dependent rats, pretreatment with prazosin prevented the increased corticosterone release during withdrawal (Fig. 1B). Administration of yohimbine before naloxone to morphine-dependent rats significantly antagonized the corticosterone hypersecretion during morphine withdrawal (Fig. 1C). Corticosterone secretion was not modified after prazosin or yohimbine injection to placebo-pelleted rats compared with the group implanted with placebo pellets receiving vehicle (Dunnett’s test).

Effects of Adrenergic Antagonists on NA Content, MHPG Production, and NA Turnover in PVN. As shown in Fig. 2A, in morphine-withdrawn rats the NA turnover increased significantly. Administration of prazosin 15 min before naloxone to morphine-dependent rats significantly antagonized that elevation in NA turnover (Fig. 2B). As shown in Fig. 2C, rats dependent on morphine receiving yohimbine prior naloxone injection did not show any alteration in NA turnover. Administration of prazosin to placebo-pelleted rats produced a significantly lower frequency of four of the seven signs (teeth-chattering, lacrimation, ptosis, and spontaneous jumping). Signs of withdrawal were not observed in the placebo groups receiving vehicle plus saline or vehicle plus naloxone. In addition, rats implanted with pellets of morphine receiving vehicle plus saline did not show any signs of abstinence.

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<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body Weight Loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plac + Veh + Sal</td>
<td>5.0 ± 0.6</td>
</tr>
<tr>
<td>Mor + Veh + Sal</td>
<td>4.9 ± 0.7</td>
</tr>
<tr>
<td>Mor + Veh + Nx</td>
<td>18.0 ± 0.6*</td>
</tr>
<tr>
<td>Plac + Praz + Sal</td>
<td>5.0 ± 0.4</td>
</tr>
<tr>
<td>Plac + Praz + Nx</td>
<td>5.5 ± 0.4</td>
</tr>
<tr>
<td>Mor + Praz + Sal</td>
<td>8.2 ± 1.3</td>
</tr>
<tr>
<td>Mor + Praz + Nx</td>
<td>12.7 ± 1.2*</td>
</tr>
<tr>
<td>Plac + Yoh + Sal</td>
<td>5.5 ± 0.5</td>
</tr>
<tr>
<td>Plac + Yoh + Nx</td>
<td>6.5 ± 0.7</td>
</tr>
<tr>
<td>Mor + Yoh + Sal</td>
<td>7.0 ± 0.8</td>
</tr>
<tr>
<td>Mor + Yoh + Nx</td>
<td>17.6 ± 0.8*</td>
</tr>
</tbody>
</table>

*Significantly different from control dependent group, χ².

Table 1:

Behavioral profiles of morphine withdrawal precipitated by naloxone

<table>
<thead>
<tr>
<th>Withdrawal Signs</th>
<th>Veh + NX</th>
<th>Pz + NX</th>
<th>Yh + NX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Teeth chattering</td>
<td>7/11</td>
<td>1/9</td>
<td>0/7*</td>
</tr>
<tr>
<td>Tremor</td>
<td>11/11</td>
<td>9/9</td>
<td>7/7</td>
</tr>
<tr>
<td>Piloerection</td>
<td>11/11</td>
<td>1/9*</td>
<td>7/7</td>
</tr>
<tr>
<td>Lacrimation</td>
<td>11/11</td>
<td>5/9*</td>
<td>2/7*</td>
</tr>
<tr>
<td>Rhinorrhea</td>
<td>11/11</td>
<td>5/9*</td>
<td>5/7</td>
</tr>
<tr>
<td>Ptosis</td>
<td>11/11</td>
<td>1/9*</td>
<td>0/7*</td>
</tr>
<tr>
<td>Spontaneous jumping</td>
<td>7/11</td>
<td>5/9</td>
<td>0/7*</td>
</tr>
</tbody>
</table>

*Significantly different from control dependent group, χ².
produced a decrease ($P < .05$) in NA turnover compared with control group receiving vehicle instead of prazosin (Dunnett's test).

Table 3 depicts NA content and MHPG production in the PVN for control rats and for rats rendered dependent on morphine and pretreated with vehicle, prazosin, or yohimbine. The morphine-pelleted group pretreated with vehicle before saline had higher levels of NA than the control placebo-pelleted rats, whereas the content of MHPG was not significantly altered. Neither placebo- nor morphine-pelleted groups showed any significant modifications in the NA or MHPG levels when prazosin was administered before saline or naloxone. In rats dependent on morphine and pretreated with yohimbine there was an increase in the NA content 30 min after saline or naloxone injection, whereas MHPG production was significantly decreased in the dependent group receiving yohimbine before saline.

Effects of Adrenergic Antagonists on DA Content, DOPAC Production, and DA Turnover in PVN. Figure 3A shows that administration of naloxone to morphine-dependent rats increased the turnover of DA in the PVN. Figure 3, B and C, depict that neither prazosin nor yohimbine significantly modified the withdrawal-induced increase in
the DA turnover. DA turnover in the placebo-pelleted group receiving prazosin or yohimbine was lower ($P < .01$) than that obtained in rats implanted with placebo pellets and injected with vehicle (Dunnnett’s test).

Table 3 shows DA and DOPAC levels in the PVN in placebo and dependent rats pretreated with vehicle, prazosin, or yohimbine. When naloxone was given to morphine-pelleted rats with vehicle, the DOPAC levels increased significantly. There were no modifications in the DA levels in any of the vehicle-pretreated groups. When prazosin was given to the morphine-dependent rats 15 min before saline, there was an increase in the DA and DOPAC levels in the PVN. However, in morphine-pelleted rats receiving prazosin before naloxone, a reduction in DA content and an elevation in DOPAC production were observed. The dependent groups receiving saline or naloxone after yohimbine showed an elevation in DOPAC levels. Placebo group receiving yohimbine before saline showed higher DA levels than the placebo-pelleted group receiving vehicle instead of yohimbine. In addition, a reduction was observed in DOPAC production in the placebo groups receiving prazosin plus saline or yohimbine plus saline compared with placebo-pelleted rats injected with vehicle before saline (Table 3).

### Discussion

Given the substantial innervation of the PVN by endogenous opioids and catecholaminergic inputs, and the proposed role for catecholamines in opioid abuse (Self and Nestler, 1995), this study was designed to investigate the possibility of a role for catecholamines in the hormonal response to morphine withdrawal. As expected, chronic morphine treatment produced physical dependence, as shown by naloxone-precipitated behavioral abstinence signs and weight loss. In addition, and consistent with previous reports (Ignar and Kuhn, 1990; González-Vez et al., 1994; Vargas et al., 1997), the present data show that naloxone-induced withdrawal produced neuroendocrine dependence, as shown by the corticosterone hypersecretion observed 30 min after the opiate antagonist injection. This alteration in the HPA axis activity was accompanied by an overproduction of the brain NA metabolite MHPG and an elevation of the MHPG/NA ratio (an index of NA turnover; Lookingland et al., 1991) in the PVN. Furthermore, the DOPAC production and the DOPAC/DA ratio, which reflects the activity of DA neurons (Manzano-Moreno et al., 1990), also were increased (Milanes et al., 1998). Because changes in catecholamine turnover were observed at the time of increased corticosterone secretion, a critical role for catecholamines in opioid-induced neuroendocrine response has been proposed (González-Vez et al., 1994; Martínez-Piñero et al., 1994; Vargas et al., 1997; Milanes et al., 1998).

The relatively high basal plasma corticosterone levels found in control rats were similar to those obtained in previous studies from our laboratory (Martínez-Piñero et al., 1994; Milanes et al., 1998). However, because animals were handled daily before the acute experiments, the potential effects of stress on the HPA axis were reduced.

The abundant noradrenergic innervation of the PVN suggests an important role for brain NA in the regulation of CRF release and in pituitary-adrenal function. In addition, evidence for a direct dopaminergic innervation of the CRF perikarya of the PVN has been provided (Liposits and Paull, 1989). Present results show that administration of adrenergic antagonists prazosin and yohimbine attenuated the behavioral signs of morphine withdrawal, suggesting a role for noradrenergic pathways in opiate dependence. Both antagonists had different effects on individual behavior: prazosin attenuated piloerection and rhinorrhea, whereas yohimbine did not. In addition, yohimbine suppressed teeth-chattering and jumping, whereas prazosin did not. Both produced a reduction in the occurrence of lacrimation and ptosis, whereas neither affected the tremor. These results are in agreement with the hypothesis that NA might play an important function in most of the signs of opiate withdrawal and clearly indicate that prazosin and yohimbine act through different adrenoceptor types (Rasmussen et al., 1990). Our results are in agreement with previous findings indicating that $\alpha_1$-agonists increase jumping during opioid withdrawal, whereas $\alpha_2$-antagonists, such as yohimbine, but not $\alpha_1$-antagonists blocked that effect (van der Laan, 1985).

Previous findings (Vargas et al., 1997; Milanes et al., 1998) and present results indicate that morphine withdrawal increases the turnover of NA and DA in the PVN of the rat concomitantly with an enhanced HPA axis activity and a decrease in CRF content in the PVN. However, it is known that administration of noradrenaline or $\alpha$-adrenergic agonists enhance the release of CRF, ACTH, and corticosterone.

### Table 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NA</th>
<th>MHPG</th>
<th>DA</th>
<th>DOPAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>plac + veh + sal</td>
<td>2943 ± 186</td>
<td>312 ± 33</td>
<td>653 ± 32</td>
<td>434 ± 32</td>
</tr>
<tr>
<td>plac + veh + nx</td>
<td>2951 ± 184</td>
<td>164 ± 32</td>
<td>770 ± 126</td>
<td>414 ± 35</td>
</tr>
<tr>
<td>mor + veh + sal</td>
<td>4143 ± 294*</td>
<td>555 ± 125</td>
<td>593 ± 68</td>
<td>363 ± 37</td>
</tr>
<tr>
<td>mor + veh + nx</td>
<td>2664 ± 191b</td>
<td>955 ± 122*</td>
<td>917 ± 111</td>
<td>900 ± 57b</td>
</tr>
<tr>
<td>plac + praz + sal</td>
<td>2512 ± 61</td>
<td>237 ± 34</td>
<td>713 ± 63</td>
<td>129 ± 14</td>
</tr>
<tr>
<td>plac + praz + nx</td>
<td>2987 ± 242</td>
<td>290 ± 53</td>
<td>594 ± 130</td>
<td>113 ± 13</td>
</tr>
<tr>
<td>mor + praz + sal</td>
<td>2326 ± 149</td>
<td>184 ± 28</td>
<td>2374 ± 253b</td>
<td>304 ± 40</td>
</tr>
<tr>
<td>mor + praz + nx</td>
<td>2155 ± 197</td>
<td>277 ± 28</td>
<td>1195 ± 215b</td>
<td>267 ± 35b</td>
</tr>
<tr>
<td>plac + yoh + sal</td>
<td>2454 ± 132</td>
<td>277 ± 38</td>
<td>1490 ± 250b</td>
<td>220 ± 20</td>
</tr>
<tr>
<td>plac + yoh + nx</td>
<td>2312 ± 210</td>
<td>180 ± 9b</td>
<td>1229 ± 202</td>
<td>215 ± 34</td>
</tr>
<tr>
<td>mor + yoh + sal</td>
<td>3832 ± 289a</td>
<td>203 ± 23a</td>
<td>1531 ± 283</td>
<td>549 ± 66a</td>
</tr>
<tr>
<td>mor + yoh + nx</td>
<td>4504 ± 362a</td>
<td>186 ± 10</td>
<td>1819 ± 224</td>
<td>706 ± 77a</td>
</tr>
</tbody>
</table>

*Significantly different versus its respective placebo-pretreated control

bSignificantly different versus respective group treated with saline s.c. (Neuman-Keuls test).

aSignificantly different versus placebo-pelleted group receiving vehicle instead prazosin or yohimbine (Dunnnett’s test).
administration of prazosin or yohimbine before naloxone injection to morphine-dependent rats antagonized the withdrawal-induced increase in the release of corticosterone. Furthermore, both antagonists totally abolished the withdrawal-induced NA turnover increase in the hypothalamic PVN. In fact, we found a direct correlation between changes in NA turnover and corticosterone response to the two \( \alpha \)-adrenoceptor antagonists prazosin and yohimbine. In contrast, present data indicate that the dopaminergic system does not participate in the hyperactivity of the HPA axis during morphine withdrawal. Although both prazosin and yohimbine decreased DOPAC production and DA turnover in placebo-pelleted rats, they did not antagonize the increased DA turnover in the PVN during morphine withdrawal.

Present data strongly suggest a role for the noradrenergic afferent innervating the hypothalamic PVN in the activation of the HPA axis during morphine withdrawal. In addition, both \( \alpha_1 \)- and \( \alpha_2 \)-receptor subtypes may be implicated in the hyperactivity of the axis occurring during morphine withdrawal because administration of \( \alpha_1 \)- or \( \alpha_2 \)-adrenoceptor antagonists blocked the increase in corticosterone secretion. A large proportion of \( \alpha_2 \)-adrenoceptor binding sites in the rat brain is postsynaptic rather than presynaptic (U’Prichard, 1984). Because both \( \alpha_1 \)- and \( \alpha_2 \)-adrenoceptors are implicated in the stimulatory effect of NA on the HPA activity, it is conceivable that the blockade of corticosterone hypersecretion produced by both prazosin and yohimbine during morphine withdrawal could be due to the blockade of postsynaptic \( \alpha_1 \)- and \( \alpha_2 \)-adrenoceptors in the PVN. However, present data show that both antagonists completely abolished the enhanced NA turnover that was seen during morphine withdrawal. It is likely, therefore, that the preventing effect of prazosin and yohimbine on corticosterone secretion during withdrawal is not produced at the PVN level. It might be the consequence of the prevention, produced by \( \alpha_1 \)- and \( \alpha_2 \)-adrenoceptors blockade, of withdrawal-induced hyperactivity in the noradrenergic pathways innervating the PVN.

The role of \( \alpha_2 \)-adrenoceptor mechanisms in withdrawal-induced hyperactivity of noradrenergic neurons and endocrine hypersecretion is difficult to determine from present results because 1) the i.c.v. injection of the \( \alpha_2 \)-agonist clonidine did not modify corticosterone hypersecretion during withdrawal (González et al., 1994); 2) there are several subtypes of \( \alpha_2 \)-adrenoceptors (\( \alpha_{2A} \), \( \alpha_{2B} \), and \( \alpha_{2C} \)), the function of which remains to be elucidated; and 3) \( \alpha_2 \)-receptors exist both pre- and postsynaptically at which sites they have very different functions. Our observation that yohimbine blocks withdrawal-induced increase in NA turnover contrasts with previous reports that the \( \alpha_2 \)-agonist clonidine reduced the activation of noradrenergic cells in the locus ceruleus (Aghanian and Wang, 1978). However, it is in agreement with previous results from our laboratory showing that clonidine did not antagonize hyperactivity of the axis during morphine withdrawal (González et al., 1994). Our paradoxical finding might be attributed to different mechanisms. First, because a number of studies have shown that \( \alpha_2 \)-receptors undergo adaptive changes in the presence of chronically administered agents that interfere with or enhance noradrenergic neurotransmission (Giralt and García-Sevilla, 1989), it is possible that yohimbine administration resulted in changes in \( \alpha_2 \)-receptor number or sensitivity, although it is difficult to predict which specific changes might result in preventing

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**Fig. 3.** Turnover of DA (as estimated by the DOPAC/DA ratio) in naive and in morphine-dependent rats 30 min after administration of saline or naloxone. The treatments were carried out as described in the legend of Table 3. Each column represents the mean ± S.E. (n = 6–13 rats per group). *, significantly different versus corresponding control placebo group; †, significantly different versus morphine-treated group receiving saline.

and also the expression of CRF mRNA in the PVN (Lookingland et al., 1991; Gunion et al., 1992; González et al., 1994; Itoi et al., 1994). Collectively, these results suggest that neuroendocrine effects of opioid withdrawal might be mediated through an increase in the release of catecholamines in the PVN. Therefore, we have examined the effects of the \( \alpha_1 \)- and \( \alpha_2 \)-adrenergic receptor blockade on the activity of the HPA axis (as measured by plasma corticosterone levels) in rats withdrawn from morphine. In parallel, we have investigated the changes in NA and DA content and turnover in the PVN after the same treatment. Present results show that...
noradrenergic hyperactivity during withdrawal. Second, an indirect action due to the disinhibition of the release of inhibitory neurotransmitters acting on NA system, such as γ-aminobutyric acid interneurons, which have been demonstrated to inhibit naloxone-induced depolarization during withdrawal (Cheng and Christie, 1996), cannot be discarded.

The site and the mechanisms underlying the prazosin prevented the increase in NA turnover in morphine withdrawn rats are not clear. A number of possible cellular and molecular mechanisms may be involved. One might include possible modulation of intracellular calcium because activation of all known α₁-adrenoceptor subtypes results in an increase of the intracellular calcium concentration (Bylund et al., 1994). This hypothesis is supported by previous findings indicating that hypothalamic NA turnover and MHPG production, both elevated during morphine withdrawal, returned to control levels in rats pretreated with the calcium channel antagonist nimodipine, concomitantly with a reduction of the corticosterone secretion (Vargas et al., 1997).

Although different α₁-receptor subtypes, such as α₁A and α₁B, have been demonstrated in the hypothalamus (Karkania et al., 1995), the α₁-blockade in the PVN does not appear to participate in the antagonistic effect of prazosin on morphine withdrawal. It appears that the ascending noradrenergic pathway to the PVN might be a candidate for mediating, at least indirectly, the effect of prazosin. Of course, it is possible that these noradrenergic afferents may be dependent on other neurotransmitter systems, which might be one of the possible sites of prazosin action.

In summary, present results confirm previous findings that dependence on morphine produces an enhanced cat

References

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