Effects of morphine withdrawal on catecholaminergic neurons on heart right ventricle; implication of dopamine receptors

M.V. Milanés, M.T. Marín, and M.L. Laorden

Abstract: The purpose of our study was to examine the effects of D1- and D2-dopamine receptors blockade on the changes in the ventricular content of catecholamines in rats withdrawn from morphine. Rats were given morphine by subcutaneous (sc) implantation of morphine pellets for 5 days. On the eighth day, morphine withdrawal was induced by sc administration of naloxone (1 mg/kg), and rats were killed 30 min later. Pretreatment with SCH 23390 (dopamine D1, D2 receptor antagonist) 15 min prior to naloxone administration suppressed some of the changes in catecholamines and dopamine turnover in the heart. By contrast, SCH 23390 did not block the hyperactivity of catecholaminergic neurons in the heart during morphine withdrawal. These results suggest that the hyperactivity of catecholaminergic neurons in the heart during morphine withdrawal is dependent upon D1 dopamine receptor activation. In addition, our results exclude the involvement of D2 dopamine receptors.

Key words: morphine withdrawal, right ventricle, catecholaminergic activity.

Résumé: La présente étude a eu pour but d’examiner les effets du blocage des récepteurs D1 et D2 de la dopamine sur les variations de la teneur ventriculaire en catécholamines chez les rats sevrés de morphine. Les rats ont reçu de la morphine sous forme d’implants s.c. pendant 5 jours. Le huitième jour, le sevrage de morphine a été induit par l’administration s.c. de naloxone (1 mg/kg); les rats ont été sacrifiés 30 min plus tard. Un traitement préalable au SCH 23390 (antagoniste des récepteurs D1 et D2 de la dopamine), 15 min avant l’administration de naloxone, a supprimé certains signes comportementaux du sevrage de morphine, alors que l’étilclopide (antagoniste des récepteurs D2, D3 et D4 de la dopamine) n’a pas eu cet effet. De plus, l’analyse biochimique indique que le SCH 23390 a complètement supprimé l’augmentation, induite par le sevrage, du renouvellement de la noradrénaline et de la dopamine dans le ventricule droit. À l’opposé, l’étilclopide n’a pas bloqué l’hyperactivité des neurones catécholaminergiques dans le cœur durant le sevrage de morphine. Ces résultats donnent à penser que l’hyperactivité des neurones catécholaminergiques dans le cœur durant le sevrage de morphine dépend de l’activation des récepteurs D1 de la dopamine. Ces résultats ne tiennent pas compte du rôle des récepteurs dopaminergiques D2.

Mots clés: sevrage de morphine, ventricule droit, activité catécholaminergique.

[Traduit par la Rédaction]

Introduction

Opioid peptides have a wide range of tissue distribution and control cardiac function through reflex mechanisms involving the central nervous system or the modulation of neurotransmitter release from neurons located within the heart (Hofaday 1983). The discovery that mammalian myocardial cells possess opioid receptors (Weihe et al. 1985; Wegener and Kummer 1994; Jin et al. 1995; Witter et al. 1996) has led to studies aimed at investigating the direct myocardial effects of opioid receptor stimulation and identifying possible intracellular opioidergic pathways.

The repeated use of opioids induces adaptive changes in the central and peripheral nervous system leading to the development to tolerance and dependence. Several animals models have been used to investigate the mechanisms involved in the responses to opioids and in the development of opiate dependence. The neurochemical mechanism involved in the development of opiate dependence and in the expression of withdrawal include homologous regulation affecting the endogenous opioid system along with heterologous regulation involving the neurotransmitter systems (Koob and Le Moal 1997). Changes in noradrenergic, dopaminergic, serotonergic, cholinergic, GABAergic, or peptidergic transmission have been reported during chronic opioid administration, and at the moment of spontaneous or naloxone-precipitated morphine abstinence (Maldonado 1997). The
rewarding properties of opiate (Wiser and Bozarth 1987) and the somatic expression of abstinence have been related to changes in mesolimbic dopaminergic activity that could constitute, at least in part, the neural substrate for opioid addiction (Koob 1992; Harris and Aston-Jones 1994). Despite substantial evidence that noradrenergic and dopaminergic neurons in the central nervous system are involved in opioid dependence and withdrawal, less information is available regarding the functional adaptive changes of heart catecholaminergic neurons during chronic exposure and upon drug withdrawal. Opioid withdrawal in the cardiovascular system is characterized by an increase in mean arterial blood pressure, biphasic heart rate response, and an increase in plasma noradrenaline (NA) and adrenaline levels (Dixon and Chandra 1987; Chang and Dixon 1990; Cruz and Villareal 1993). In addition, previous studies in our laboratory demonstrated that the acute administration of naloxone in morphine-treated rats produced an increase in the turnover of dopamine (DA) in cardiac tissues, which could be responsible for the increase in the force and frequency of contraction observed in vitro experiments (Rabadán et al. 1997). However, the exact mechanism involved in heart catecholaminergic neurons during chronic morphine treatment and upon drug withdrawal have not been clarified, and there are not conclusive results. Because dopamine receptors have been implicated in the behavioural responses to drug abuse (Harris and Aston-Jones 1994; Koob 1996; Uhl et al. 1998), the purpose of our study was to elucidate the involvement of dopamine receptor subtypes in morphine withdrawal. To accomplish this, injections of (R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride (SCH 23390), an antagonist selective for the dopamine D₁ receptor subfamily (D₁, D₃), and eticlopride, an antagonist selective for the dopamine D₂ receptor subfamily (D₂, D₃, D₄), were administered to naive and morphine-dependent rats before naloxone administration. The content of NA, DA, and their metabolites in the right ventricle were measured to investigate if changes in catecholaminergic turnover during morphine withdrawal are modified by dopamine receptor manipulation.

### Material and methods

**Male Sprague-Dawley rats weighing 200–220 g (at the beginning of the experiments) were housed four to five per cage under a 12 h light : dark cycle in a room with controlled temperature (22 ± 1°C) and humidity (50 ± 10%). Food and water were available ad libitum. The animals were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care.**

**Experimental procedure**

On the basis of previous studies (González et al. 1994; Rabadán et al. 1997; Vargas et al. 1997), rats were implanted with morphine base pellets (75 mg) subcutaneously under light ether anaesthesia as follows: one on day 1, two on day 3, and three on day 5. The pellets were implanted under sterile conditions. This pelleting method provides continuous exposure to morphine and has been shown to induce both tolerance and dependence as measured behaviourally and biochemically (Couceyro and Douglas 1995; Rabadán et al. 1997; Vargas et al. 1997). Control animals were implanted with placebo pellets containing lactose using the same time schedule. On day 8 animals were injected i.p with vehicle, SCH 23390 (0.25 mg/kg) or eticlopride (0.1 mg/kg). Fifteen minutes later they received saline (sc) or naloxone (1 mg/kg sc) and then were observed for behavioural signs of withdrawal. The doses of SCH 23390 and eticlopride used in the present study have been shown to block all subtypes in the D₁ and D₂ family and antagonize several effects of morphine (Roquerbet et al. 1992; Jezierski and White 1995).

The incidence of teeth clattering, piloerection, lacrimation, rhinorhea, spontaneous jumping, tremor, and ptosis were scored for 30 min. These behavioural signs are reliable markers of opioid withdrawal in morphine-dependent rats and have previously been employed as indices of the degree of dependence (Maldonado et al. 1992). At the end of this period animals were killed, and analytical studies were conducted. The experimental groups were: placebo plus vehicle, SCH 23390 or eticlopride plus saline (control); placebo plus vehicle, SCH 23390, or eticlopride plus naloxone (naloxone control); morphine plus vehicle, SCH 23390, or eticlopride plus saline (chronic morphine treatment); and morphine plus vehicle, SCH 23390, or eticlopride plus naloxone (naloxone-precipitated withdrawal).

Weight gain was checked during treatment to assure that morphine was liberated correctly from the pellets, since chronic morphine treatment induces a decrease in body weight gain as a result of lower caloric intake (Berhow et al. 1995). In addition, on the day of experiment weight loss was calculated as the difference between the weight determined immediately before the saline or naloxone injection and a second determination made 30 min later, immediately before death.

**Analytical procedure for estimation of ventricular catecholamines**

After decapsulation, the chest was opened with a midsternal incision and the right ventricle was dissected and stored immediately at −80°C until assayed for NA, its metabolite normetanephrine (NMN), DA, and its metabolite 3,4-dihydroxyphenyl acetic acid (DOPAC) by high performance liquid chromatography (HPLC) with electrochemical detection. Each tissue was weighed, placed in a dry-cooled propylene vial, and homogenized with a Polytron-type homogenizer (setting 4 for 40 s) in 1.5 mL perchloric acid (0.1 M). The homogenates were then centrifuged (20 000 rpm; 4°C, 15 min), the supernatant layer was removed into a 1-mL syringe and filtered through a 0.45-μm filter (Millipore, Bedford, Mass.) and centrifuged (15 000 rpm, 4°C, 20 min) again through Ultraflute MC 0.2 (Millipore). Ten microlitres of each sample was injected into a 5-μm C₁₈ reverse-phase column (Waters Associates, Millipore Corp., Bedford, Mass.) 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 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 analyzed with Millenium 2010 Chromatography Manager (Millipore) equipment. DOPAC, NA, NMN, and DA were simultaneously detected by the described HPLC method at elution times 3.12, 4.00, 7.00, and 10.70 min, respectively. NA, DA, 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 NA, DA, NMN, and DOPAC in the right ventricle was expressed as ng/g weight of tissue.

### Drug and chemicals

Pellets of morphine base (Alcaliber Labs., Madrid, Spain) or lactose were prepared by the Department of Pharmacy and Phar-
maceutical Technology (School of Pharmacy, Granada, Spain). NA bitartrate, NMN, DA HCl, DOPAC (used as HPLC standards) naloxone, and naloxone HCl were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). SCH 23390 and eticlopride were purchased from Research Biochemical Incorporated (Mass., U.S.A.). Naloxone HCl and SCH 23390 were dissolved in sterile 0.9% NaCl (saline) and eticlopride was dissolved in deionized water. Drugs were freshly prepared every day. Other reagents were of analytical grade.

### Statistical analysis

The data are expressed as mean ± SEM. The significance of the differences in the contents of NA, NMN, DA, DOPAC, and in the NMN/NNA and DOPAC/DA ratios were determined by analysis of variance (ANOVA) followed by the Newman-Keuls test, using a computer programme. The nonpaired Student’s t test was used when comparing the means of body weight change. Behaviours were quantified as the number of animals exhibiting the sign/total number of animals observed, and data obtained were analysed nonparametrically using the $\chi^2$ test. The significance level was taken as $p < 0.05$.

### Results

Weights were recorded on the day of pellet implantation and day of decapitation (day 8), before receiving any injection. In all experimental groups, rats chronically treated with morphine showed significantly ($p < 0.001$) lower body weight gain (21.66 ± 1.32 g) than animals receiving placebo pellets (52.46 ± 1.14 g) (data not shown). The regimen of morphine pellet implantation produced dependence, as showed by the ability of naloxone to precipitate standard signs of withdrawal. Naloxone (1 mg/kg) caused characteristic abnormal behaviour signs, such as teeth-chattering, tremor, piloerection, lacrimation, rhinorrhoea, ptosis, and spontaneous jumping. Significant total suppression of two of the seven signs (teeth-chattering and piloerection) was noted in the dependent group pretreated with SCH 23390 before naloxone injection. However, pretreatment with eticlopride before naloxone did not change the standard signs of withdrawal (Table 1).

As shown in Fig. 1A, administration of naloxone to control rats resulted in no significant change in body weight loss when measured 30 min after the drug injection. However, chronic morphine-treated animals showed significant weight loss ($p < 0.001$) 30 min after naloxone injection, when compared with the morphine-treated group injected with saline or the placebo group injected with naloxone. In morphine-dependent rats pretreated with SCH 23390 or eticlopride there were also a significant ($p < 0.001$) weight loss 30 min after naloxone injection when compared with the tolerant groups receiving saline instead of naloxone or with the placebo group injected with naloxone (Fig. 1B, 1C).

### Effects of dopaminergic antagonists on NA, NMN content, and NA turnover in the right ventricle

Table 2 depicts NA and NMN content in the right ventricle for control rats and for rats rendered dependent on morphine and treated with vehicle, SCH 23390, or eticlopride before saline or naloxone. Neither placebo- nor morphine-pelleted groups showed any significant modifications in the NA content when naloxone or saline were administered. However, the content of NMN was increased ($p < 0.05$) in

### Table 1. Behavioural profiles of morphine withdrawal precipitated by naloxone (1 mg/kg, sc).

<table>
<thead>
<tr>
<th>Withdrawal signs</th>
<th>veh + nx</th>
<th>SCH + nx</th>
<th>etc + nx</th>
</tr>
</thead>
<tbody>
<tr>
<td>teeth chattering</td>
<td>7/11</td>
<td>0/7**</td>
<td>6/6</td>
</tr>
<tr>
<td>tremor</td>
<td>11/11</td>
<td>6/7</td>
<td>6/6</td>
</tr>
<tr>
<td>piloerection</td>
<td>11/11</td>
<td>0/7**</td>
<td>6/6</td>
</tr>
<tr>
<td>lacrimation</td>
<td>11/11</td>
<td>7/7</td>
<td>6/6</td>
</tr>
<tr>
<td>rhinorrhoea</td>
<td>11/11</td>
<td>7/7</td>
<td>6/6</td>
</tr>
<tr>
<td>ptosis</td>
<td>11/11</td>
<td>6/6</td>
<td>6/6</td>
</tr>
<tr>
<td>spontaneous jumping</td>
<td>7/11</td>
<td>4/7</td>
<td>6/6</td>
</tr>
</tbody>
</table>

**Note:** Rats were injected with vehicle (veh) ip, SCH 23390 (0.25 mg/kg, ip), or eticlopride (0.1 mg/kg, ip, etc) 15 min before naloxone (nx) administration. Rats were observed for 30 min for signs of dependence. These behaviours are shown as the number of animals exhibiting the signs to the total number of animals observed. *$p < 0.01$, **$p < 0.001$, significantly different from control dependent group, $\chi^2$.

the morphine-pelleted rats after naloxone injection versus the naive rats injected with naloxone and the dependent group receiving saline (Table 2). As shown in Fig. 2A, in rats undergoing withdrawal from repeated morphine treatment by naloxone injection, the NA turnover (as estimated by NMN/NNA ratio) increased significantly ($p < 0.01$) when compared with the naive rats injected with naloxone and with the dependent group receiving saline.

The administration of SCH 23390 to placebo- or morphine-pelleted rats before saline or naloxone did not induce significant changes in the levels of NA and NMN (Table 2). In addition, in morphine-dependent rats receiving SCH 23390 before naloxone no modification in the NMN/NNA ratio was observed (Fig. 2B). Similarly, the levels of NA were not modified in the naive or dependent rats injected with eticlopride before saline or naloxone. However, the NMN levels were increased in the morphine-dependent rats treated with eticlopride before naloxone versus the placebo group injected with naloxone ($p < 0.05$) and the morphine-dependent group injected with saline ($p < 0.05$) (Table 2). As shown in Fig. 2C, rats rendered dependent on morphine receiving eticlopride before naloxone injection showed a significant increase ($p < 0.01$) in the NMN/NNA ratio when compared with the dependent group injected with saline and the placebo group injected with naloxone ($p < 0.01$).

### Effects of dopaminergic antagonists on DA, DOPAC content, and DA turnover in the right ventricle

Table 2 shows the DA and DOPAC levels in the right ventricle in placebo and dependent rats treated with vehicle, SCH 23390, or eticlopride before saline or naloxone. When naloxone was given to morphine-pelleted rats treated with vehicle, the DOPAC levels increased significantly when compared with its placebo control group ($p < 0.01$) and with the dependent group injected with saline instead of naloxone ($p < 0.05$) (Table 2). Figure 3A shows that administration of naloxone to morphine-dependent rats treated with vehicle increased the DOPAC/DA ratio (as index of DA turnover) versus the placebo group injected with naloxone ($p < 0.01$) and the dependent group injected with saline ($p < 0.05$).

The administration of SCH 23390 to the naive or morphine-dependent rats 15 min before saline or naloxone did not cause significant changes in the DA or DOPAC levels, or

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Table 2. Effect of pretreatment with SCH 23390 (0.25 mg/kg, ip) or eticlopride (0.1 mg/kg, ip) on NA, NMN, DA, and DOPAC levels in the right ventricle of naïve (plac) and morphine (mor)-dependent rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NA (ng/g)</th>
<th>NMN (ng/g)</th>
<th>DA (ng/g)</th>
<th>DOPAC (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>plac+veh+sal</td>
<td>1063 ± 86.0</td>
<td>22.11 ± 2.7</td>
<td>24.2 ± 1.9</td>
<td>7.8 ± 2.3</td>
</tr>
<tr>
<td>plac+veh+nx</td>
<td>970 ± 88.9</td>
<td>24.5 ± 3.5</td>
<td>29.0 ± 4.4</td>
<td>5.7 ± 0.6</td>
</tr>
<tr>
<td>mor+veh+sal</td>
<td>756 ± 49.5</td>
<td>36.3 ± 8.2</td>
<td>18.2 ± 0.9</td>
<td>8.6 ± 2.3</td>
</tr>
<tr>
<td>mor+veh+nx</td>
<td>795 ± 45.0</td>
<td>58.0 ± 8.7**</td>
<td>20.0 ± 1.7</td>
<td>17.8 ± 3.9**</td>
</tr>
<tr>
<td>plac+sch+sal</td>
<td>989 ± 4.8</td>
<td>25.6 ± 1.5</td>
<td>22.2 ± 2.0</td>
<td>8.0 ± 0.2</td>
</tr>
<tr>
<td>plac+sch+nx</td>
<td>820 ± 60.6</td>
<td>22.6 ± 0.9</td>
<td>22.5 ± 1.2</td>
<td>7.5 ± 0.6</td>
</tr>
<tr>
<td>mor+sch+sal</td>
<td>791 ± 31.6</td>
<td>28.2 ± 8.2</td>
<td>21.3 ± 3.1</td>
<td>9.3 ± 1.0</td>
</tr>
<tr>
<td>mor+sch+nx</td>
<td>874 ± 94.4</td>
<td>25.8 ± 1.2</td>
<td>24.0 ± 0.3</td>
<td>10.0 ± 1.1</td>
</tr>
<tr>
<td>plac+etc+sal</td>
<td>822 ± 53.9</td>
<td>20.2 ± 3.6</td>
<td>24.1 ± 2.3</td>
<td>5.9 ± 0.9</td>
</tr>
<tr>
<td>plac+etc+nx</td>
<td>898 ± 95.7</td>
<td>19.4 ± 2.4</td>
<td>24.5 ± 3.2</td>
<td>6.5 ± 0.6</td>
</tr>
<tr>
<td>mor+etc+sal</td>
<td>1033 ± 45.9</td>
<td>17.4 ± 2.0</td>
<td>26.5 ± 4.0</td>
<td>10.8 ± 2.3</td>
</tr>
<tr>
<td>mor+etc+nx</td>
<td>1053 ± 66.0</td>
<td>50.3 ± 9.0**</td>
<td>23.3 ± 3.3</td>
<td>20.0 ± 3.0***</td>
</tr>
</tbody>
</table>

Note: SCH 23390, eticlopride (etc), or vehicle (veh, ip) were administered 15 min before saline (sal) or naloxone (nx). Testing occurred 30 min after saline (sc) or naloxone (1 mg/kg sc). Data are the means ± SEM. n = 6–11 for each experimental group. *p < 0.05, **p < 0.01, ***p < 0.001 vs. its respective placebo-pretreated control; *p < 0.05, **p < 0.01, ***p < 0.001 vs. respective group treated with saline sc.

in the DA turnover (Table 2, Fig. 3B). The administration of eticlopride to naïve or dependent rats before naloxone or saline did not induce any changes in the DA levels, whereas DOPAC levels was increased in the morphine-dependent group injected with naloxone versus the placebo group injected with naloxone (p < 0.001) and the dependent group injected with saline (p < 0.01) (Table 2). In addition, the morphine-dependent rats pretreated with eticlopride before naloxone showed an increase (p < 0.001) in the DOPAC/DA ratio when compared with the dependent group injected with saline or the naïve rats injected with naloxone (Fig. 3C).

Discussion

As expected, our results show that chronic morphine treatment by pellet implantation produced physical dependence, as demonstrated by naloxone-precipitated behavioural abstinence signs and weight loss. In addition, our data show that naloxone-induced withdrawal produced an increase in NMN levels and in the NMN ratio (an index of NA turnover, Milanés and Laorden 1998) in the right ventricle. Furthermore, the DOPAC levels and the DOPAC/DA ratio, which reflect the activity of DA neurons (Manzanares et al. 1990), were also increased. This agrees with previous studies in the right and left atri (Rabadán et al. 1997a, 1998). Since opioids interact with dopamine systems, we were interested in determining the role of D₃ and D₄ dopamine receptors on morphine withdrawal in the heart.

Physical dependence associated with chronic morphine treatment is characterized by a withdrawal syndrome comprising various specific behavioural signs which occur after abrupt cessation of treatment or after administration of an opioid antagonist (Lookingland et al. 1991). Our results show that administration of SCH 23390 or eticlopride had different effects on individual behaviour. Tremor, lacrimation, rhinorrea, ptosis, and spontaneous jumping were observed after naloxone injection to tolerant rats pretreated with both antagonists; however, the pretreatment with SCH 23390 suppressed teeth chattering and piloerection while eticlopride pretreatment did not. These data indicate that dopamine D₃ receptors are not involved in the manifestation of the somatic signs of opioid withdrawal. In agreement with this finding, it has been demonstrated that the presence of dopamine D₃ receptors are not required to obtain behaviour signs of withdrawal in morphine-dependent animals (Zarrindast and Farzin 1996; Maldonado et al. 1997).

Previously, studies from this laboratory (Rabadán et al. 1997a, 1998; Milanés and Laorden 2000; Milanés et al. 2000) showed that morphine withdrawal increases the turnover of NA and DA in the heart. The present results confirm this finding and clearly indicate that morphine withdrawal increases NA and DA turnover, suggesting that noradrenergic and dopaminergic pathways are involved in the hyperactivity of the autonomic nervous system associated with morphine withdrawal in the heart.

Several studies in the central nervous system indicate that the dopaminergic system exerts an important control on the opioid withdrawal phenomenon. However, as far as we know, the possible role of heart dopamine receptors in morphine withdrawal has never been investigated. Therefore, we examined the effects of the D₃ and D₄ dopaminergic receptor blockade on changes in the ventricular content of catecholamines in rats experiencing morphine withdrawal. The existence of dopamine receptor subtypes D₁ (predominantly localized at the postsynaptic sites; Oozono et al. 1996) and D₂ (present at presynaptic sites; Goldberg and Kohli 1983; Hilditch and Drew 1985; Ameta et al. 1993) in the heart of mammalian species, including humans, is well established. In addition, it has been suggested the expression of a dopamine D₃ receptor occurs in rat atria but not in ventricles (Ricci et al. 1998).

Our results show that the administration of SCH 23390 prior to naloxone injection to morphine-dependent rats abolished the withdrawal-induced NA and DA turnover increase in the right ventricle, whereas eticlopride did not affect the increase of NA and DA turnover observed during morphine withdrawal. This suggests that mainly D₃ dopamine receptor subtypes are involved in the control of morphine withdrawal. These findings are consistent with several studies in the cen-
Fig. 1. Changes in body weight in naive and in morphine-dependent animals. Rats were given placebo or morphine pellets for 5 days. On day 8, groups of rats were pretreated with (A) vehicle (veh, ip), (B) SCH 22390 (0.25 mg/kg, ip), or (C) eticlopride (etc, 0.1 mg/kg, ip) 15 min before naloxone (nx, 1 mg/kg, sc) administration. Data represent weight loss 30 min after naloxone injection (n = 6–11 per group; means ± SEM). +++p < 0.001 vs. placebo group injected with naloxone; +++p < 0.001 vs. morphine-dependent rats injected with saline instead of naloxone.

Fig. 2. Turnover of NA (as estimated by the NMN/NA ratio) in naive and in morphine-dependent rats 30 min after administration of saline or naloxone. The treatment were carried out as described in the legend of Fig. 1. Each column represents the mean ± SEM. n = 6–11 experiments. +++p < 0.01 vs. placebo + veh + nx; **p < 0.01 vs. morphine + veh + saline (sal).

The results of our study indicate marked receptor selectivity in the heart for dopamine-mediated modulation of morphine withdrawal, which involved D₁ but not D₂ receptors. There is a possible explanation for this selectivity: D₁ and D₂ dopamine receptors are coupled to adenylate cyclase but the stimulation of D₁ receptors causes an increased production of 3'-5'-cyclic adenosine monophosphate (cAMP), whereas stimulation of D₂ receptors causes a decrease of nucleus accumbens has been observed in morphine withdrawal; this decrease does not appear to be crucial to the morphine withdrawal syndrome, but may instead represent an adaptive mechanism to oppose its aversive symptoms (Georges et al. 1999).
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 Fig. 1. Each column represents the mean ± SEM. n = 6–11 experiments. +++p < 0.001 vs. placebo + veh + nx; *p < 0.05, ***p < 0.001 vs. morphine + veh + saline (sal).

On the other hand, a previous study in our laboratory has demonstrated that the administration of quaternary opioid antagonists, which do not cross the blood-brain barrier, to rats dependent on morphine induced an increase in NA and DA turnover (Milánés et al. 2001). In addition, in situ intrinsic cardiac neurons can function independent of central neuronal input (Huang et al. 1993). These data suggest that the changes observed during morphine withdrawal in the heart are due to an intrinsic mechanism outside the central nervous system, so the involvement of central D₁ receptors is ruled out.

In conclusion, our data indicate that the dopaminergic system exerts an important control on the opioid withdrawal phenomenon. The inhibition of hyperactivity of the catecholaminergic pathways by SCH 23390 suggest that the dopamine D₁ receptor is involved in morphine withdrawal in the heart. The blockade of this receptor will perhaps allow new perspectives for the therapy of morphine withdrawal syndrome.

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