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Changes in catecholaminergic pathways innervating the rat heart ventricle during morphine dependence. Involvement of α_1- and α_2-adrenoceptors

M. Victoria Milanés, M. Luisa Laorden *

Department of Physiology and Pharmacology, Unit of Pharmacology. University School of Medicine, Murcia, Spain

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Abstract

In the present study, we examined the effects of α_1- and the α_2-adrenoceptors blockade on the changes in the ventricular content of catecholamines in rats withdrawn from morphine. Rats were given morphine by s.c. implantation of morphine pellets for 5 days. On the seventh day, morphine withdrawal was induced by s.c. administration of naloxone (1 mg/kg), and rats were killed 30 min later. Pretreatment with yohimbine (α_2-adrenoceptor) or prazosin (α_1-adrenoceptor) 15 min prior to naloxone administration attenuated some of the behavioural signs of morphine withdrawal. In addition, biochemical analysis indicated that yohimbine completely abolished the withdrawal-induced increase in noradrenaline and dopamine turnover in the right ventricle. By contrast, prazosin did not block the hyperactivity of catecholaminergic neurons in the heart during withdrawal. These data suggest that the hyperactivity of catecholaminergic neurons in the heart during morphine withdrawal is dependent upon α_2-adrenoceptor activation. In addition, the present results rule out the involvement of α_1-adrenoceptors.

Keywords: Morphine withdrawal; Ventricle, right; Catecholaminergic activity; Yohimbine; Prazosin

1. Introduction

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

The repeated use of opioids induces adaptative changes in the central and peripheral nervous system leading to the development of tolerance and dependence. Several animal models have been used to investigate the mechanisms involved in the responses to opioids and in the development of tolerance and dependence (Nestler et al., 1993). Although the mechanisms underlying the development of physical dependence and the expression of symptoms of the abstinence syndrome are not clear, indirect evidence suggests that multiple mechanisms may be operating in these processes (Koob, 1992). Thus, changes in catecholaminergic, serotoninergic, cholinergic, gamma aminobutyric acid (GABA)-ergic or peptidergic transmission have been reported during chronic opioid administration and at the moment of spontaneous or naloxone-precipitated morphine abstinence (Maldonado, 1997). The central noradrenergic system seems to have an important role in the development of physical dependence on opioid receptor agonists and in the expression of withdrawal signs (Nestler, 1992; Nestler et al., 1993). This involvement is supported by the biochemical changes reported in noradrenergic transmission during opioid dependence and withdrawal, and on the pharmacological response induced by opioid withdrawal elicited by the administration of adrenergic compounds (Maldonado, 1997). Despite the

* Corresponding author. Departamento de Farmacología, Facultad de Medicina, Campus de Espinardo, 301000 Murcia, España. Tel.: +34-968-367155; fax: +34-968-364150.

E-mail address: laorden@fcu.um.es (M.L. Laorden).

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substantial evidence that catecholaminergic neurons in the central nervous system are involved in opioid dependence and withdrawal, less information is available regarding the functional adaptative changes of heart catecholaminergic neurons during chronic opioid exposure and upon drug withdrawal. It has been suggested that the injection of naloxone to rats pretreated with morphine precipitates a withdrawal response including an increase in mean arterial blood pressure, biphasic heart rate response and an increase in plasma noradrenaline and adrenaline levels (Chang and Dixon, 1990; Dixon and Chandra, 1987; Cruz and Villareal, 1993). In addition, previous studies in our laboratory have demonstrated that the acute administration of naloxone in morphine-treated rats produces an increase in the turnover of dopamine in auricular tissues, an increase which could be responsible for the increase in the force and frequency of contraction observed in vitro experiments (Rabadán et al., 1998, 1997b). However, the exact mechanisms involved in the adaptive changes in heart catecholaminergic neurons during chronic morphine treatment and upon drug withdrawal have not been clarified, and there are no conclusive results. Therefore, the purpose of this study was to elucidate the involvement of α-adrenoceptors in opioid dependence and withdrawal. To accomplish this, yohimbine (α_2-adrenoceptor antagonist) and prazosin (α_1-adrenoceptor antagonist) were injected in naïve and morphine-dependent rats before naloxone administration and the content of noradrenaline, dopamine and their metabolites in the right ventricle was measured to investigate whether the changes in catecholaminergic turnover during dependence are modified by α-adrenoceptor manipulation.

2. Material and methods

Male Sprague–Dawley rats (weight 200–220 g at the beginning of experiments) were housed four to five per cage under a 12-h light/dark cycle in a room with controlled temperature (22 ± 11°C) and humidity (50 ± 10%) and with food and water available ad libitum. The investigation conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985). These investigations were carried out under the approval of a local ethics committee.

2.1. Experimental procedure

On the basis of previous studies (Gonzálvez et al., 1994; Vargas et al., 1997; Rabadán et al., 1998, 1997b), rats were 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 anaesthesia. This pelleting method provides continuous exposure to morphine and has been shown to induce both the tolerance and dependence as measured behaviourally and biochemically (Couceyro and Douglass, 1995; Rabadán et al., 1997a; Vargas et al., 1997). Control animals were implanted with placebo pellets containing lactose according to the same time schedule. On day 7, animals were injected i.p. with vehicle, yohimbine (2 mg/kg) or prazosin (1 mg/kg) and 15 min later received saline (s.c.) or naloxone (1 mg/kg s.c.), and were then observed for behavioural signs of withdrawal. The incidence of teeth chattering, piloerection, lacrimation, rhinorrhea, spontaneous jumping, tremor and ptosis was scored for 30 min. These behavioural 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, the animals were killed, and analytical studies were conducted. The 12 experimental conditions were: placebo plus vehicle or yohimbine or prazosin plus saline (control); placebo plus vehicle or yohimbine or prazosin plus naloxone (naloxone control); morphine plus vehicle or yohimbine or prazosin plus saline (chronic morphine treatment) or morphine plus vehicle or yohimbine or prazosin plus naloxone (naloxone-precipitated withdrawal).

The rats, weight gain was checked during treatment in order to confirm that morphine was liberated correctly from the pellets, since it is known that chronic morphine treatment induces a decrease in body weight gain due to a lower caloric intake. In addition, on the day of the 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 killing.

2.2. Analytical procedure for estimation of ventricular catecholamines

After decapitation, the chest was opened with a midsternal incision and the right ventricle was dissected and

Table 1
Behavioural profiles of morphine withdrawal precipitated by 1 mg/kg^{-1} s.c. naloxone (nx). Rats were injected with vehicle (veh) i.p., yohimbine (2 mg/kg i.p.; yh) or prazosin (1 mg/kg i.p.; pz) 15 min before naloxone administration. Rats were observed for 30 min for signs of dependence. These behaviours are shown as the number of animals exhibiting the signs out of the total number of animals observed

Withdrawal signs	veh + nx	yh + nx	pz + nx
Teeth-chattering	7/11	0/7**	1/9
Tremor	11/11	7/7	9/9
Piloerection	11/11	7/7	1/9*
Lacrimation	11/11	2/7**	5/9***
Rhinorrhea	11/11	5/7	5/9***
Ptosis	11/11	0/7***	1/9***
Spontaneous jumping	7/11	0/7*	5/9

*$P < 0.05$, significantly different from control dependent group, χ^2.
**$P < 0.01$, significantly different from control dependent group, χ^2.
***$P < 0.001$, significantly different from control dependent group, χ^2.

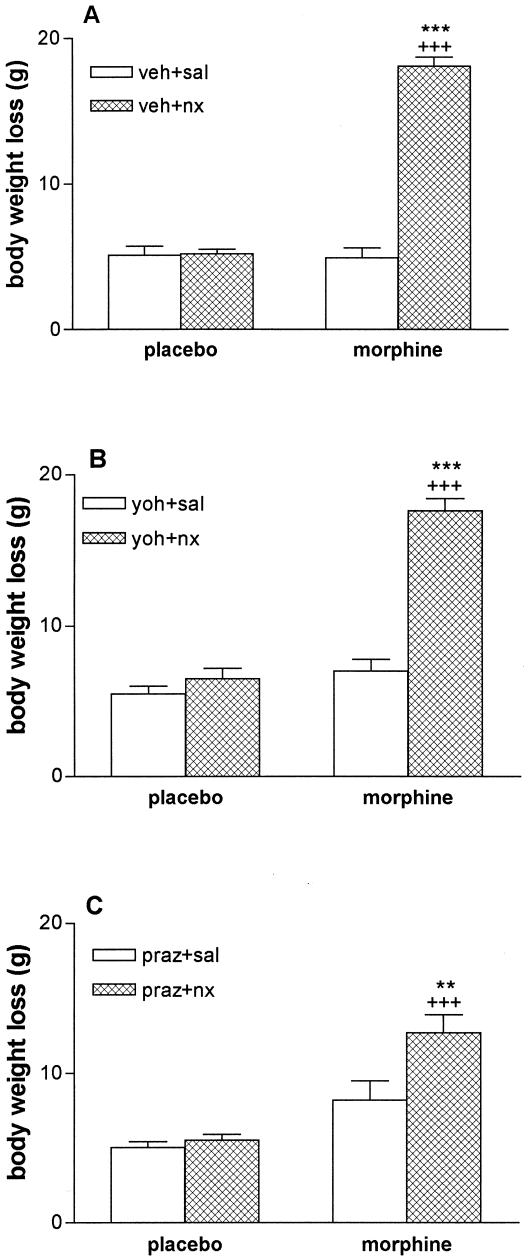

Fig. 1. Changes in body weight in naïve and in morphine-dependent rats. Rats were given placebo or morphine pellets for 5 days. On day 7, groups of rats were pretreated with vehicle (veh, i.p.) (A), yohimbine (2 mg/kg, yoh, i.p.) (B) or prazosin (1 mg/kg, praz, i.p.) (C) 15 min before naloxone (1 mg/ kg, nx, s.c.) administration. Data represent weight loss 30 min after naloxone injection ($n = 7–11$ per group; mean ± s.e.). $^{++}P < 0.001$ vs. placebo-treated rats injected with naloxone; $^{**}P < 0.01$, $^{***}P < 0.001$ vs. morphine-dependent rats injected with saline instead of naloxone.

stored immediately at −80°C until assayed for catecholamines. Noradrenaline and its metabolite normetanephrine, dopamine and its metabolite 3,4-dihydroxyphenyl acetic acid (DOPAC) were determined by high-performance liquid chromatography with electrochemical detection (HPLC-ED). Each tissue was weighed, placed in a dry-cooled propylene vial and homogenized with a Polytron-type homogenizer (setting four for 40 s) in 1.5 ml perchloric acid (0.1 M). The homogenates were then centrifuged (20.000 rpm; 4°C, 15 min), and the supernatant layer was removed into a 1-ml syringe and filtered through a 0.45-μm filter (Millipore) and centrifuged (15.000 rpm, 4°C, 20 min) again through Ultrafree MC 0.2 (Millipore). Ten microlitres of each sample was injected into a 5-μm C_{18} reverse-phase column (Waters) through a Rheodyne syringe-loading injector (200 μl loop). Electrochemical de-

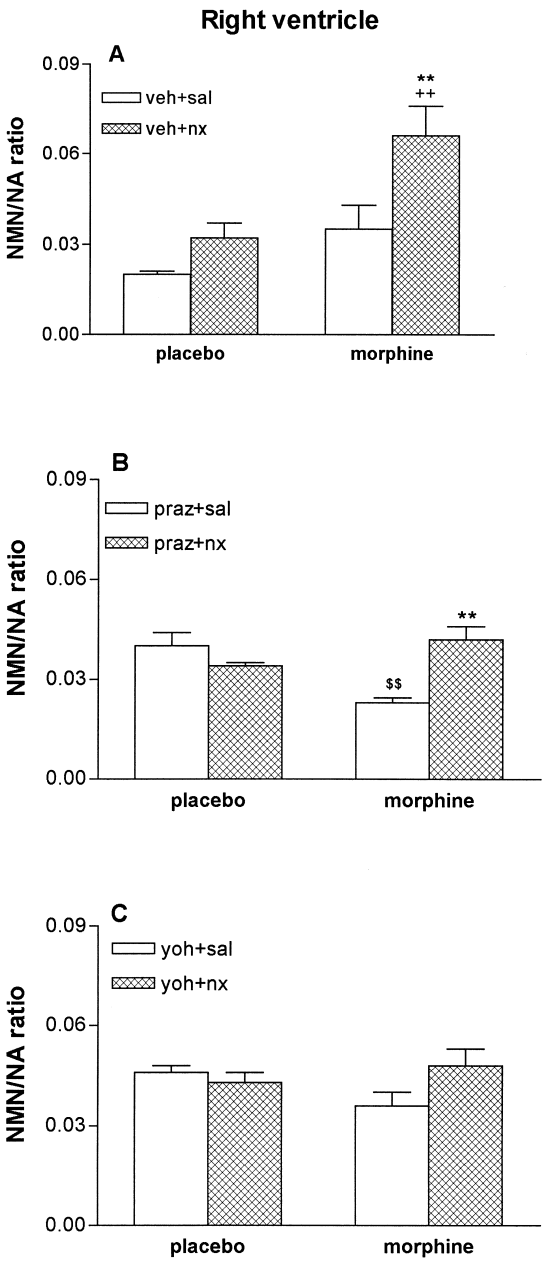

Fig. 2. Turnover of NA (as estimated by the NMN/NA ratio) in naïve and in morphine-dependent rats 30 min after administration of saline or naloxone. The treatments were carried out as described in the legend of Fig. 1. Each column represents the mean ± s.e.m. of 6–9 experiments. $^{++}P < 0.01$ vs. placebo + veh + saline (sal); $^{**}P < 0.01$ vs. morphine + veh + naloxone (nx); $^{\$\$}P < 0.01$ vs. placebo + praz + sal.

tection was accomplished with a glassy carbon electrode set at a potential of +0.65 V vs. the Ag/AgCl reference electrode (Waters). The mobile phase consisted of a 95:5 (v/v) mixture of water and methanol with sodium acetate (50 mM), citric acid (20 mM), 1-octyl-sodium sulphonate (3.75 mM), di-*n*-butylamine (1 mM) and EDTA (0.135 mM), adjusted to pH 4.3. The flow rate was 0.9 ml/min and chromatographic data were analysed with Millennium 2010 Chromatography Manager (Millipore) equipment. DOPAC, noradrenaline, normetranephrine and dopamine were simultaneously detected by the described HPLC method at elution times of 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.

2.3. 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), noradrenaline bitartrate, normetanephrine, dopamine HCl and DOPAC (used as HPLC standards). Naloxone HCl, yohimbine HCl and prazosin HCl were purchased from Sigma Chemical (St. Louis, MO, USA). Naloxone HCl was dissolved in sterile 0.9% NaCl (saline) and yohimbine and prazosin were dissolved in sterile distilled water. Drugs were prepared freshly every day. Other reagents were of analytical grade.

2.4. Statistical analysis

The data are expressed as means ± S.E.M. The significance of the differences in the contents of noradrenaline, normetanephrine, doapmine, DOPAC and in the normetanephrine/noradrenaline and DOPAC/dopamine ratios was determined by analysis of variance followed by the Newman–Keuls' test by using a computer program. Non-paired 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 non-parametrically using the χ^2 test. Significance level was taken as $P < 0.05$.

3. Results

The regimen of 5 days of morphine pellet implantation produced dependence, as shown by the ability of naloxone to elicit standard signs of withdrawal. Naloxone (1 mg/kg s.c.) caused characteristic signs of abnormal behaviour, such as teeth-chattering, tremor, piloerection, lacrimation, rhinorrhea, ptosis and spontaneous jumping. A significantly lower frequency or total suppression of four of the seven signs (teeth-chattering, lacrimation, ptosis and spontaneous jumping) was noted in the dependent group pretreated with yohimbine before naloxone injection. Pretreatment with prazosin before naloxone also produced a lower frequency of four of the seven signs (piloerection, lacrimation, rhinorrhea and ptosis) (Table 1).

As shown in Fig. 1, administration of naloxone (1 mg/kg) to control rats resulted in no significant changes

Table 2
Effects of pretreatment with yohimbine (2 mg/kg i.p.) or prazosin (1 mg/kg i.p.) on NA, NMN, DA and DOPAC content in the right ventricle of naïve (plac) and morphine (mor)-dependent rats. Yohimbine (yoh), prazosin (praz) or vehicle (veh, i.p.) was administered 15 min before saline (sal) or naloxone (nx). Testing occurred 30 min after saline (s.c.) or naloxone (1 mg/kg s.c.). Data are the means ± s.e.m. for *n* rats (6–9)

Treatment	NA (ng/g)	NMN (ng/g)	DA (ng/g)	DOPAC (ng/g)
plac + veh + sal	1063.0 ± 86.0	22.1 ± 2.7	24.2 ± 1.9	7.8 ± 2.3
plac + veh + nx	970.0 ± 88.9	24.5 ± 3.5	29.0 ± 4.4	5.7 ± 0.6
mor + veh + sal	756.0 ± 49.5	36.3 ± 8.2	18.2 ± 0.9	8.6 ± 2.3
mor + veh + nx	795.9 ± 45	58.0 ± 8.7$^{*,\#}$	20.0 ± 1.7	17.8 ± 3.9$^{**,\#}$
plac + yoh + sa	927.8 ± 111.2	43.0 ± 5.5	53.0 ± 2.0	4.4 ± 0.6
plac + yoh + nx	1131.0 ± 83.2	51.5 ± 4.7	45.8 ± 3.8	9.4 ± 3.0
mor + yoh + sal	911.0 ± 78.9	33.6 ± 2.1	70.6 ± 4.4$^{\#\#\#}$	9.3 ± 1.0
mor + yoh + nx	1412.0 ± 107.0$^{\#}$	48.4 ± 5.5	104.3 ± 4.9$^{***,\#\#}$	8.4 ± 1.7
plac + praz + sal	1414.0 ± 106.9	45.0 ± 2.5	42.0 ± 4.1	16.8 ± 1.0
plac + praz + nx	1242.0 ± 73.3	38.7 ± 2.1	41.8 ± 5.0	13.7 ± 1.2
mor + praz + sal	1344.0 ± 120.2	38.4 ± 4.5	80.2 ± 3.4†	19.0 ± 0.3
mor + praz + nx	775.7 ± 39.2$^{***,\#\#}$	34.5 ± 3.0	47.7 ± 6.2$^{\#\#}$	20.8 ± 1.6**

$^{*}P < 0.05$ vs. its respective placebo-pretreated control.
$^{**}P < 0.01$ vs. its respective placebo-pretreated control.
$^{***}P < 0.001$ vs. its respective placebo-pretreated control.
$^{\#}P < 0.05$ vs. respective group treated with saline s.c.
$^{\#\#}P < 0.001$ vs. respective group treated with saline s.c.
$^{\#\#\#}P < 0.01$ vs. its respective placebo-pretreated group injected with naloxone.
$^{\dagger}P < 0.001$vs. its respective placebo-pretreated group injected with naloxone.

in body weight loss when measured 30 min after the drug injection. However, chronic morphine-treated animals showed an important weight loss ($P < 0.001$) 30 min after naloxone injection, when compared with the morphine-treated rats injected with saline s.c. In morphine-dependent rats pretreated with yohimbine or prazosin, there was also a significant weight loss 30 min after naloxone injection when compared with that of morphine-treated rats injected with saline or control rats injected with naloxone.

3.1. Effects of adrenoceptor antagonists on noradrenaline, normetanephrine content and noradrenaline turnover in the right ventricle.

As shown in Fig. 2A, in rats undergoing naloxone-induced withdrawal from repeated morphine treatment, noradrenaline turnover (as estimated by the normetanephrine/noradrenaline ratio) increased significantly when compared with that of control rats injected with naloxone and with dependent rats receiving saline. However, in morphine-dependent rats receiving yohimbine before naloxone injection, no modification of the normetanephrine/noradrenaline ratio was observed (Fig. 2C). As shown in Fig. 2B, rats rendered dependent on morphine receiving prazosin before naloxone injection showed a significant increase in the normetanephrine/noradrenaline ratio when compared with the dependent rats injected with saline. There was a reduction of noradrenaline turnover in the dependent rats receiving prazosin + saline compared with that of the control rats (placebo + prazosin + saline).

Table 2 depicts the noradrenaline and normetanephrine content in the right ventricle for control rats and for rats rendered dependent on morphine and pretreated with vehicle, yohimbine or prazosin. Neither control nor morphine-dependent rats showed any significant modifications in the noradrenaline content when naloxone was administered. However, the content of normetanephrine was higher in the morphine-dependent rats after naloxone injection than that in the control rats injected with naloxone or the dependent rats receiving saline. In rats dependent on morphine and pretreated with yohimbine or prazosin, there was an increase in the noradrenaline content 30 min after naloxone injection, when compared with that in the dependent rats injected with saline, whereas the normetanephrine levels were not altered.

3.2. Effects of adrenoceptor antagonists on dopamine, DOPAC content and dopamine turnover

Fig. 3A shows that administration of naloxone to morphine-dependent rats increased the DOPAC/dopamine ratio (as index of dopamine turnover) compared with that of control rats injected with naloxone and the dependent rats injected with saline. Fig. 3C shows that yohimbine did not significantly affect the withdrawal-induced increase in dopamine turnover. However, the morphine-dependent rats

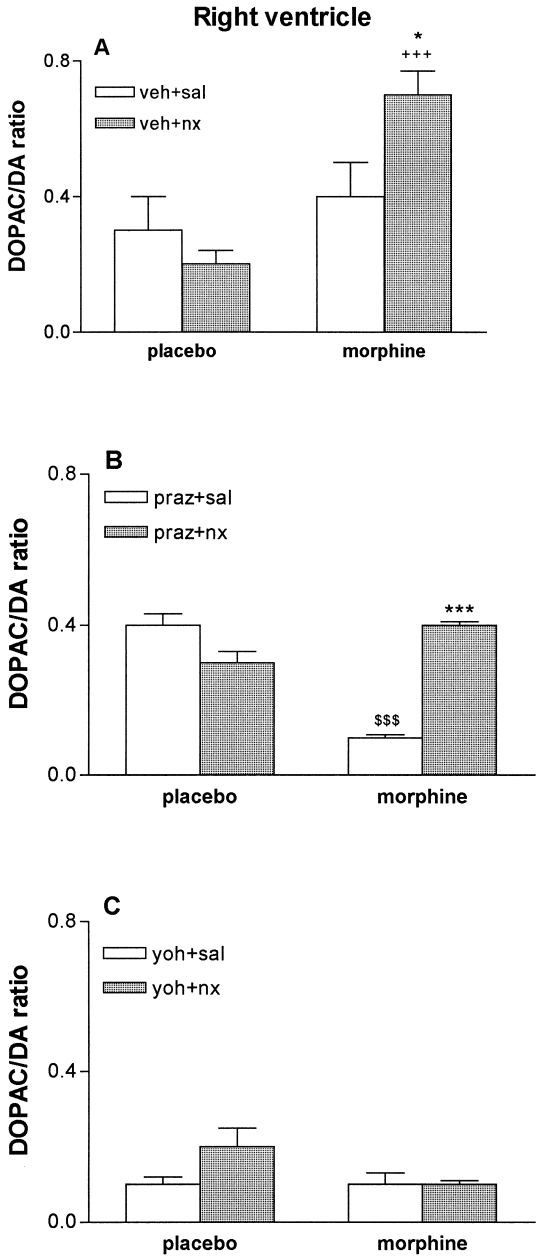

Fig. 3. Turnover of DA (as estimated by the DOPAC/DA ratio) in naïve and in morphine-dependent rats 30 min after administration of saline or naloxone. The treatments were carried out as described in the legend of Fig. 1. Each column represents the mean ± s.e.m. of 6–9 experiments. $^{+++}P < 0.001$ vs. placebo + veh + saline (sal); $^{*}P < 0.05$, $^{***}P < 0.001$ vs. morphine + veh + nx; $^{\$\$\$}P < 0.01$ vs. placebo + prazosin (praz) + sal.

pretreated with prazosin before naloxone showed an increase of the DOPAC/dopamine ratio when compared with the control rats injected with naloxone, whereas the turnover of dopamine was decreased in the dependent rats injected with saline when compared with that of control rats injected with saline (Fig. 2B).

Table 2 shows the dopamine and DOPAC levels in the right ventricle in control and morphine-dependent rats pretreated with vehicle, yohimbine or prazosin. When

naloxone was given to morphine-dependent rats pretreated with vehicle, the DOPAC levels increased significantly when compared with those of the control rats and the morphine-dependent rats injected with saline instead of naloxone. There was no modification of the dopamine level in any of the vehicle-pretreated rats. When yohimbine was given to the morphine-dependent rats 15 min before saline, there was a significant increase in the dopamine levels when compared with those of the control rats injected with saline. The administration of naloxone to dependent rats pretreated with yohimbine produced a significant increase in the dopamine levels compared with those of the control rats injected with naloxone and the morphine-dependent rats injected with saline without any changes in the DOPAC content. In the control rats pretreated with prazosin, there were no changes in the dopamine and DOPAC content. However, the morphine-dependent rats injected with saline showed an increase in dopamine levels compared with the control rats injected with saline. The administration of naloxone to the morphine-dependent rats pretreated with prazosin induced a decrease in dopamine levels compared with those of the dependent rats injected with saline. In addition, the dependent rats receiving naloxone after prazosin showed a decrease in the levels of DOPAC compared with control rats injected with naloxone.

4. Discussion

As expected, the present results show that chronic morphine treatment by pellet implantation produced physical dependence, as indicated by the reduction in body weight increase and the behavioural signs of abstinence. In addition, the present data show that naloxone-induced withdrawal produced an increase in the normetanephrine levels and in normetanephrine/noradrenaline ratio, an index of noradrenaline turnover (Milanés and Laorden, 1998) in the cardiac ventricle. Furthermore, the DOPAC levels and the DOPAC/DA ratio, which reflect the activity of DA neurons (Manzanares et al., 1990), were also increased as well, as shown previously (Rabadán et al., 1998, 1997b).

Physical dependence associated with chronic morphine treatment is characterized by a withdrawal syndrome comprising various specific behavioural signs which occur after abrupt cessation (Lookingland et al., 1991) of treatment or after administration of an opioid receptor antagonist. The present results show that administration of the adrenergic antagonists yohimbine and prazosin attenuated the behavioural signs of morphine withdrawal, suggesting a role for adrenergic pathways in opiate dependence. The two antagonists had different effects on individual behaviours: both reduced the occurrence of lacrimation and ptosis, while neither affected the tremor. However, prazosin attenuated piloerection and rhinorrhea, while yohimbine did not. In addition, yohimbine suppressed teeth-chattering and jumping, while prazosin did not. Since a strong correlation has been shown between morphine withdrawal behaviour and activation of noradrenergic pathways, the present results are in agreement with the hypothesis that noradrenaline might play an important function in most of the signs of opiate withdrawal (Rasmussen et al., 1990).

Previous findings (Rabadán et al., 1997b, 1998; Milanés et al., 2000) and the present results indicate that morphine withdrawal increases the turnover of noradrenaline and dopamine in the right ventricle, which suggests that catecholaminergic pathways are involved in the hyperactivity of the autonomic nervous system associated with morphine withdrawal. Therefore, we examined the effects of α_1- and α_2-adrenergic adrenoceptor blockade on the changes in the ventricular content of catecholamines in rats undergoing withdrawal from morphine. The existence of α_1-adrenoreceptors, predominantly localized at the post-synaptic level, and α_2-adrenoceptors, present at pre-synaptic sites, in the heart of most mammalian species, including humans, is well established (Schüman, 1980; Kenneth and Szilagyl, 1992; Blaxal et al., 1994; Michel et al., 1994). The present results show that administration of yohimbine prior to naloxone injection to morphine-dependent rats abolished the withdrawal-induced increase in noradrenaline and dopamine turnover in the right ventricle. The involvement of α_2-adrenoceptors in opiate dependence and withdrawal has been mainly suggested due to the anti-withdrawal effects of α_2-adrenoceptor agonists. Thus, it has been demonstrated that clonidine reduces the autonomic manifestations and the intensity of most of the somatic symptoms of withdrawal (Buccafusco et al., 1984; Tierney et al., 1988; Kelsey et al., 1990).

The role of α_2-adrenoreceptor mechanisms in withdrawal-induced hyperactivity of catecolaminergic neurons in the right ventricle is difficult to determine from the present results because: (i) there are several subtypes of α_2-adrenoceptors (α_{2A}, α_{2B}, α_{2C}), the function of which remains to be elucidated, and (ii) α_2-adrenoreceptors exist both pre- and post-synaptically, at which sites they have very different functions. Our observation that yohimbine blocked the withdrawal-induced increase in noradrenaline and dopamine turnover contrasts with the results of a previous study of the central nervous system in which clonidine reduced the activation of noradrenergic cells in the locus coeruleus (Rasmussen et al., 1990; Baraban et al., 1995), although it is in agreement with previous results from our laboratory showing that clonidine does not antagonize hyperactivity of the axis during morphine withdrawal (Gonzálvez et al., 1994). In addition, it has been demonstrated that α_2-adrenoceptor antagonist yohimbine prevents the development of morphine dependence (Taylor et al., 1991; Iglesias et al., 1992; Ambrosio et al., 1997; Laorden et al., 2000).

Our findings might be attributed to different mechanisms: (i) since a number of studies have shown that

α_2-adrenoceptors undergo adaptive changes in the presence of chronically administered agents that interfere with or enhance noradrenergic neruotransmission (García-Sevilla et al., 1985; Giralt and García-Sevilla, 1989; Smith et al., 1989; Busquets et al., 1997), it is possible that yohimbine administration results in changes in α_2-adrenoceptor number or sensitivity, although it is difficult to predict which specific changes prevent noradrenergic hyperactivity during withdrawal; (ii) other mechanism could be related with intracellular Ca^{2+} levels. Thus, a suggested mechanism linked with α_2-adrenoceptor activation is an increased intracellular availability of calcium (Taylor et al., 1991; Aantaa et al., 1995), which is involved in neurotransmitter release. Therefore, yohimbine might act through this proposed mechanism, blocking the increase in intracellular calcium levels necessary to release catecholamines.

However, α_1-adrenoceptors have also been proposed to participate in some components of withdrawal. Thus, the administration of phentolamine, phenoxybenzamine and prazosin (Cicero et al., 1974; Van der Laan, 1999) decreases the incidence of some signs of opiate withdrawal. The administration of dapiprazole, another α_1-adrenoreceptor antagonist, prior to morphine has been reported to block the development of acute opiate dependence (Valeri et al., 1988) observed during morphine withdrawal. The present data demonstrated that prazosin decreased the incidence of some signs of withdrawal. However, it did not affect the increase of noradrenaline and dopamine turnover observed during morphine withdrawal. These results suggest that post-synaptic α_1-adrenoreceptors are not involved in the catecholaminergic modifications seen during opioid withdrawal, which is in agreement with the idea that the regulation of post-synaptic α_1-adrenoceptor regulation is of secondary importance in morphine-dependence in the heart.

In summary, these results confirm previous findings showing that dependence on morphine produces an enhanced turnover of noradrenaline and dopamine in the heart and suggest that the hyperactivity of the catecholaminergic pathway in the heart could be mediated through α_2-adrenoceptors. The involvement of α_1-adrenoceptors can be ruled out since prazosin did not modify the increase in noradrenaline and dopamine turnover observed during morphine withdrawal.

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