Effects of Chronic Morphine Treatment on Catecholamines Content and Mechanical Response in the Rat Hearts

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ABSTRACT
Our investigation was aimed at elucidating if the chronic administration and withdrawal of a preferential μ-agonist, morphine, induce changes on the heart catecholaminergic neuronal activity. With this purpose the effects of morphine or naloxone (preferentially μ-antagonist) on noradrenaline, adrenaline and dopamine (DA) content and the mechanical response of the left atria were studied in chronically placebo- or morphine-treated rats (implanted s.c. with pellets for 7 days). In morphine-treated rats, a challenge dose of morphine (30 mg/kg i.p.) increased the auricular noradrenaline, adrenaline and DA content and decreased dihydroxy phenyl acetic acid/DA ratio; these changes were accompanied by a decrease in the force of contraction in the isolated left atria. No changes were observed in placebo-treated rats. The administration of naloxone (1 mg/kg s.c.) to morphine-treated animals induced a decrease on the auricular content of noradrenaline, adrenaline and DA and an increase in dihydroxy phenyl acetic acid/DA ratio. The study of the mechanical response to naloxone in the isolated left atria showed an enhancement in the force of contraction in preparations from morphine-treated rats, whereas in the placebo-pelleted rats naloxone induced a decrease in this parameter. These findings demonstrate that the heart of rats that had received chronic morphine-treatment exhibit excitatory reactions to naloxone-precipitated withdrawal and suggest that the changes observed in the heart by the chronic administration of morphine and after naloxone precipitated withdrawal are mostly mediated by the catecholaminergic system.

It is well established that chronic administration of opioids results in the development of tolerance to their pharmacological actions. Physical dependence on opioids also develops after repeated administration, but its intensity depends on the specific type of opioid agonist used (Bhargava, 1991). In general drugs acting at the μ-opioid receptors, such as morphine and heroin, are highly addicting, whereas those acting at the κ-site produce a very mild degree of physical dependence (Cowan and Murray, 1990). Several attempts have been made to ascertain the possible role of catecholamines in the genesis and/or expression of tolerance and dependence processes. Thus, in naïve rats, the hypothalamic content of NA and DA was reduced after acute administration of morphine, which suggests that the stimulation of opioid receptors produces an increase in the release of these amines in the hypothalamus (Milanés et al., 1993). An increased turnover in hypothalamic NA and DA neurons is routinely observed after morphine administration (Althee et al., 1989). In contrast, the morphine-induced reduction in the hypothalamic NA and DA concentrations was inhibited in chronically morphine-treated rats, which suggests that tolerance is developed to the effect of the opiate in noradrenergic and dopaminergic neurons (Milanés et al., 1993). Recently, it has been demonstrated that the administration of naloxone in placebo-treated animals increased the hypothalamic content of NA. However, in chronically morphine-treated rats, naloxone reduced the hypothalamic noradrenaline content (González et al., 1994). In addition, the precipitation of withdrawal in morphine-dependent rats by administration of opiate antagonists caused an elevation in firing of NA neurons in the locus coeruleus and an increase in the turnover of NA in the forebrain as well as a behavioral syndrome that has been associated with these effects (Aghajaniam, 1978; Rasmussen et al., 1990).

Despite it being well known that catecholamines play a significant role in opioid tolerance and dependence in central nervous system very little is available on the cardiovascular changes during morphine tolerance or dependence. Studies suggest that chronic administration of U-50,488H induced tolerance in cardiac functions, which was not accompanied by down-regulation of κ-binding sites (Xia et al., 1994). In addition, a few studies indicated that catecholamines play an important role in the sympathetic manifestation of the abstinence response in morphine-dependent rats (Chang and Dixon, 1990; Cruz and Villarrreal, 1993).

ABBREVIATIONS: NA, noradrenaline; A, adrenaline; DA, dopamine; DOPAC, dihydroxy phenyl acetic acid; DHBA, 3,4-dihydroxy-benzylamine; MPLM, myenteric plexus-longitudinal muscle.
Our study was undertaken to determine whether the chronic administration and withdrawal of a preferential \(\mu\)-agonist, morphine, produces changes in the content of NA, A and DA in the left atria. In addition, it was determined whether such changes are correlated with alterations in the force of auricular contraction in the isolated left atria of the rat.

**Methods**

**Animals.** Male Sprague-Dawley rats (200–250 g) were housed four to five per cage under a 12-hr light/dark cycle (light 08:00–20:00), in a room with controlled temperature (22 \(\pm\) 1°C) and humidity (50 \(\pm\) 10%), and food and water available *ad libitum*.

**Drugs.** Pellets of morphine base (Alcaliber Labs., Madrid, Spain) or lactose were prepared by the Department of Pharmacy, Clinic Hospital (Madrid, Spain). Reserpine base (Sigma Chemical Co., England) was dissolved in distilled water containing (\(\sqrt{\text{v/v}}\) 2% of benzyl alcohol polysorbato 80 (10%) and citric acid (250 mg). Morphine HCl (Alcaliber Labs.) and naloxone HCl (Merck, Sharp & Dome, Madrid, Spain) were prepared fresh every day, dissolved in sterile 0.9% NaCl (saline) or distilled water.

**Chronic treatment with morphine.** Morphine tolerance was induced by the s.c. implantation, under light ether anesthesia, of pellets containing morphine base (75 mg). The implantation schedule consisted of one pellet on day 0, two pellets on day 2 and 3 pellets on day 4. Control groups received placebo pellets (lactose) according to the same time schedule. Animals were killed on day 7 (08:30–09:00). With this dosage schedule, rats show complete tolerance to the hypothermic and neuroendocrine effects of morphine (Martinez et al., 1990). On day 7 rats were treated acutely with saline i.p. or morphine (30 mg/kg i.p.) and killed 30 min later. To determine whether catecholamine secreting neurons were involved in the morphine effects, rats were pretreated with reserpine (5 mg/kg i.p.), a depletor of catecholamines or vehicle (control) and 18 hr later animals received injections with morphine (30 mg/kg i.p.) or saline i.p. and killed 30 min after.

A withdrawal syndrome was induced by injecting naloxone (1 mg/kg s.c.) on the morning they were killed (day 7) to rats implanted with morphine pellets. The control group was implanted with placebo pellets and injected with naloxone. Animals were killed 30 min after naloxone injection.

There were 12 experimental groups: 1) chronic placebo-acute saline i.p.; 2) chronic placebo-acute morphine i.p.; 3) chronic morphine-acute saline i.p.; 4) chronic morphine-acute morphine i.p.; 5) chronic placebo-acute saline s.c.; 6) chronic placebo-acute naloxone s.c.; 7) chronic morphine-acute saline s.c.; 8) chronic morphine-acute naloxone s.c.; 9) chronic placebo-vehicle of reserpine-acute saline i.p.; 10) chronic placebo-reserpine-acute morphine i.p.; 11) chronic morphine-vehicle of reserpine-acute saline i.p. and 12) chronic morphine-reserpine-acute morphine i.p.

**Analytical procedure for estimation of auricular catecholamines.** NA, A, DA and DOPAC were estimated in the left atria of the rat by high performance liquid chromatography with electrochemical detection (HPLC/ED, Waters Millipore, MA). After decapitation, the heart was opened with a midsternal incision and the left atria was dissected and stored at \(-80°C\) until assayed for catecholamines. The left atria was weighed and immediately placed in a dry ice-cooled polypropylene vial and was homogenized with a Polytron homogenizer (setting 5 for 30 sec) in 1 ml perchloric acid (0.1 M) containing EDTA (2.7 mM/liter) and 3,4 dihydroxy-benzylamine (DHBA 5 pg/µl; Waters) as internal standard. The homogenates were centrifuged \((15,000 \text{ rpm}, 4°C, 15 \text{ min})\), the supernatant layer was removed into a 1-ml syringe and filtered through a 0.22 µm GV (Millipore) and 10 µl of each simples was injected into 5-µm C18 reserve-phase column (Waters). Electrochemical detection was accomplished with a glassy carbon electrode set at a potential of +0.65 V vs. the Ag/AgCl reference electrode. 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 sulfonate [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. Chromatographic data were analyzed with a Waters 740 Date Module integrator and quantified using the peak area ratio of the internal standard. Auricular content of catecholamines was expressed as ng/g wet weight of tissue.

**Isolated left atria preparation.** The left atria was isolated and suspended in a 10 ml organ bath. Tyrode solution of the following composition (mM) was used: NaCl 136.9; KCl 5.0; MgCl\(_2\) 1.05; CaCl\(_2\) 1.8; NaHPO\(_4\) 0.4; NaHCO\(_3\) 11.9; dextrose 5.0. The bathing solution was maintained at 37°C, pH 7.4 and bubbled with 95% O\(_2\) and 5% CO\(_2\). Atria was electrically stimulated with a Grass SD-9 stimulator by means two platinum ring electrodes with rectangular pulses at a frequency of 0.2 Hz, duration of 5 msec and supramaximal voltage (35 V). Each preparation was suspended under a resting tension of 0.5 g and equilibrated for 45 min before the start of the experiments. Electrically induced contractions were measured by using a force-displacement transducer (Grass FT-03) and recorded on a Letica polygraph. Only preparations that had a stable basal contractile activity at the end of the stabilization period were accepted for study.

**Atrial responses to morphine and naloxone.** Concentration-response curves (10\(^{-8}\)–10\(^{-4}\) M) to morphine were made in the isolated left atria from chronically morphine-treated rats and from the respective controls chronically treated with lactose pellets. To evaluate whether the response to morphine is mediated by the release of catecholamines, animals were pretreated with reserpine (5 mg/kg i.p., 1 hr before the experiments) or vehicle. Thus, there were the following experimental groups: 1) placebo pellets-morphine HCl, 2) morphine pellets-morphine HCl, 3) placebo pellets + vehicle of reserpine-morphine HCl and 4) placebo pellets + reserpine-morphine HCl, 5) morphine pellets + vehicle of reserpine-morphine HCl and 6) morphine pellets + reserpine-morphine HCl.

Each drug concentration was added to the organ bath in a volume of 0.1 ml in a cumulative manner. Concentration of drugs were increased after a steady-state response had been attained with the previous concentrations or after 5 min in the absence of response.

Concentration response curves for naloxone (10\(^{-3}\)–10\(^{-6}\) M) were made in the left atria from rats chronically treated with morphine or placebo pellets. Concentration of the drugs were increased after a steady-state response had been attained with the previous concentrations.

**Statistical analysis.** The data are expressed as means \(\pm\) S.E.M. Statistical differences in the content of NA, A, DOPAC and DOPAC/DA ratio were performed by two-way analysis of variance followed by the Tukey post hoc test. Results obtained *in vitro* are expressed as fractions of the change in force of contraction produced by a maximum dose of morphine or naloxone (mean of 6 animals \(\pm\) S.E.M.). In this case statistical analysis was performed by one-way analysis of variance followed by the Tukey post hoc test. Differences with P < .05 were considered significant.

**Results**

**Effects of chronic morphine treatment on catecholamines content in left atria.** In rats implanted with placebo pellets, morphine (30 mg/kg) did not modify the contents of NA (834.0 \(\pm\) 27.8 ng/g), A (27.6 \(\pm\) 3.1 ng/g) or DA (33.2 \(\pm\) 2.4 ng/g) in left atria of the rat when compared to those in saline-treated rats (868.6 \(\pm\) 63.8, 26.75 \(\pm\) 6.0, 41.75 \(\pm\) 7.3 ng/g, respectively) (figs. 1, A–C). In the same group of animals morphine neither change the content of DOPAC nor the DOPAC/DA ratio (table 1). However, in morphine-treated rats the acute administration of morphine significantly increased the tissue levels of NA (1458.2 \(\pm\) 128.3 ng/g; P < .001), A (67.5 \(\pm\) 3.3 ng/g; P < .01) or DA (40.0 \(\pm\) 3.2...
ng/g; P < .05) as compared to its respective saline-injected control groups (829.0 ± 75.9, 39.5 ± 1.4, 24.0 ± 0.9 ng/g, respectively) (fig. 1, A–C). In addition, the DOPAC/DA ratio were significantly (P < .01) reduce in morphine-treated animals receiving injections with morphine vs. saline group (table 1).

The results on the effect of reserpine on the tissue levels of catecholamines contents are shown in figure 2, A–C. There was a drastic decrease (P < .01; P < .001) in the contents of NA, A and DA in the left atria after morphine administration in placebo- (8.6 ± 0.1, 8.3 ± 0.7 and 7.7 ± 0.1 ng/g, respec-

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<th>Table 1: Concentrations of DOPAC and ratio DOPAC/DA in left atria 30 min after morphine (30 mg/kg i.p.) or saline administration in rats chronically treated with placebo or morphine pellets</th>
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Mean ± S.E.M. of 5 to 6 for each experimental group.

a P < .01 vs. morphine + saline.
b P < .05 vs. placebo + morphine.

Discussion

Different methods have been used in several studies to induce opioid tolerance. In our study, the method of morphine pellet implantation and the schedule used were similar to that previously described (Martinez et al., 1990), which produces a high degree of tolerance to the effects of morphine at the central nervous system. Moreover, opioid tolerance
and withdrawal syndrome can be demonstrated in isolated tissues, using the same time schedule, such as MPLM strips from guinea pig (Collier et al., 1981; Garaulet et al., 1994a; Garaulet et al., 1994b; Garaulet et al., 1995). However, few studies have been performed in cardiac tissues (Xia et al., 1994). Therefore, the mechanism involved in opioid tolerance and dependence in the heart are poorly understood.

In our study the concentration-response curves with morphine on isolated left atria were begun approximately 1 hr after isolation of the tissue and were undertaken in tissues that were set up in opioid-free Tyrode's solution. No differences in the degree of tolerance or dependence have been observed in preparations that were maintained in morphine or were examined in the absence of the opioid (Johnson, 1991).

Our data show that administration of morphine to placebo-pelleted rats did not alter the auricular content of NA, A and DA. In addition, the auricular force of contraction did not change in preparations from placebo-treated rats. According to our data, previous results have shown that high concentrations of morphine are need to induce cardiac electrophysi-

### Figures

**Fig. 2.** Auricular NA (A), A (B) and DA (C) concentrations in placebo-(Pla) and morphine- (Mor) pelleted rats treated with reserpine (res, 5 mg/kg i.p.) or vehicle (veh) 30 min after acutely injected saline i.p. or morphine (30 mg/kg i.p.). Each bar represents the mean ± S.E.M. of five to six experiments. ***P < .001 vs. placebo + vehicle; ++P < .01; +++P < .001 vs. morphine + vehicle.

**Fig. 3.** Auricular NA (A), A (B) and DA (C) concentrations in placebo- and morphine-pelleted rats treated with naloxone (1 mg/kg s.c.) or saline s.c. Each bar represents the mean ± S.E.M. of five to six experiments. *P < .05; **P < .01 vs. placebo + saline; +++P < .001 vs. placebo + naloxone.
was observed, which could not account for the extent of tolerance observed (Xia et al., 1994). In addition, we have demonstrated that chronic treatment with morphine or sufentanil resulted in tolerance on MPLM to U-50,488H, and chronic treatment with U-50,488H resulted in tolerance to morphine and to DAMGO (Garaulet et al., 1994a, 1995). These data suggest that during tolerance the opioid-binding sites were unchanged, so is likely that tolerance resulted from reduced effectiveness of the transduction mechanisms.

Another objective of this work was to explored if naloxone-precipitated withdrawal in morphine-treated rats is mediated by catecholamines. Our data show that in morphine-treated rats, the acute administration of naloxone decreased the auricular content of NA, A and DA, whereas the ratio DOPAC/DA was increased. However, in placebo-treated rats, naloxone did not alter the auricular content of NA, A or the ratio DOPAC/DA. Moreover, in preparations from morphine-treated rats naloxone induced a dose-dependent increase in the force of contraction. In contrast, in preparations from placebo-treated rats the antagonist decreased this parameter. These data demonstrated that naloxone-induced withdrawal is characterized by an increase in the release and turnover of catecholamines, which is accompanied by an enhancement of the force of contraction. These results agree with previous studies (Dixon and Chandra, 1987; Chang and Dixon, 1990; Cruz and Villareal, 1993) confirming that catecholamines play an important role in the manifestation of the abstinence response at the heart level. Parallel studies on opioid dependence in central nervous system demonstrated that after naloxone administration to morphine-treated rats, animals displayed all behavioral signs and symptoms of opioid withdrawal and enhanced plasma corticosterone levels, simultaneously with a reduction in the hypothalamic content of NA (González et al., 1994). The reduction in noradrenaline content is consistent with an increase in its turnover (Gabriel et al., 1985; Taylor et al., 1988). Also, studies in guinea pig ileum MPLM showed similar response for precipitated abstinence (Garaulet et al., 1994b). Thus, naloxone induced a withdrawal contracture on MPLM from morphine-treated animals, which is due to an increase in the release of acetylcholine. Our results extend the findings in the MPLM to a different animal species, to a different physiological
integrative level and to a different chain of neurotransmission systems.

In conclusion, the present results suggest that the changes observed by chronic administration of morphine and after withdrawal in the heart are mostly mediated by the catecholaminergic system. These data may be important to understand the changes induced in the mechanical response in the heart in subjects dependent on opioid who received morphine or opioid antagonists.

References


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