Changes in right atrial catecholamine content in naïve rats and after naloxone-induced withdrawal

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Summary

In this study, we determined if changes in heart catecholamine content during naloxone-induced withdrawal correlated with modifications in heart rate. In addition, we determined plasma concentrations of corticosterone as an index of the hypothalamo–pituitary–adrenal (HPA) axis. The effects of naloxone on norepinephrine, epinephrine or dopamine content and turnover, plasma concentrations of corticosterone and the mechanical response of the right atria of the rat were studied. Male rats were implanted with placebo or morphine pellets for 7 days. On the day of sacrifice, animals were injected with saline or naloxone 1 mg kg⁻¹ s.c. to precipitate a withdrawal syndrome. Administration of naloxone to morphine-treated (tolerant) animals induced a decrease in atrial content of norepinephrine, epinephrine and dopamine (290.2 (11.9) ng g⁻¹, 15.6 (2.1) ng g⁻¹ and 9.52 (0.5) ng g⁻¹, respectively) and an increase (1.38 (0.2) ng g⁻¹ in the dihydroxy phenyl acetic acid/dopamine (DOPAC/DA) ratio. Administration of naloxone to morphine-treated animals enhanced plasma concentrations of corticosterone (435.8 (27.6) ng ml⁻¹). In the isolated right atria, L-naloxone induced an increase in atrial rate in preparations from morphine-treated rats whereas in placebo-pelleted (naïve) rats, L-naloxone induced a decrease. In contrast, administration of D-naloxone (inactive isomer) produced a decrease in atrial rate in preparations from placebo or morphine-treated rats. We conclude that this study has provided evidence that naloxone-induced withdrawal was characterized by activation of catecholaminergic neurones in the heart that was accompanied by an increase in atrial rate. (Br. J. Anaesth. 1998; 80: 354–359)

Keywords: heart, heart rate; sympathetic nervous system, catecholamines; antagonists opioid, naloxone; rat

The mechanisms by which opioid tolerance and dependence develop are still not clearly understood. Different mechanisms have been sought to explain the development of opioid tolerance–dependence and the effects on drug withdrawal, including adaptative changes in neurotransmitter systems that appear to be targets of opioids (for review see Nestler4). Several attempts have been made to ascertain the possible role of catecholamines in the genesis or expression of dependence processes. The noradrenergic system has been demonstrated to play an important role in opioid dependence and withdrawal,3 and studies of opioid regulation of the noradrenergic system have focussed mainly on the locus coeruleus. Thus precipitation of withdrawal in morphine-dependent rats by administration of opioid antagonists caused an increase in firing of norepinephrine neurones in the locus coeruleus and an increase in the turnover of norepinephrine in the forebrain, in addition to a behavioural syndrome that has been associated with these effects.6

Also, it has been proposed that opioids can affect the activity of the hypothalamo–pituitary–adrenal (HPA) axis indirectly by altering noradrenergic neurotransmission10 and attempts have been made to relate opioid-induced changes in hypothalamic noradrenergic activity to alterations in corticosterone secretion (as a marker of the HPA axis).9,10 Moreover, it has been demonstrated that chronic administration of morphine increases the hypothalamic content of norepinephrine. However, during naloxone-induced withdrawal, the hypothalamic content of norepinephrine decreased and was accompanied by a parallel increase in plasma concentrations of corticosterone.11,12 There is substantial evidence that noradrenergic neurones in the locus coeruleus and hypothalamus are involved in opioid dependence and withdrawal,11,12 yet little data are available on the cardiovascular changes during morphine tolerance or dependence. It has been suggested that chronic administration of U-50,488H induced tolerance to cardiac function, which was not accompanied by down-regulation of κ binding sites.13 In addition, a few studies indicated that catecholamines play an important role in the manifestation of the abstinence response in morphine-dependent rats. Thus adminis-
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As the different symptoms of withdrawal may involve the central and peripheral nervous systems, in the present study we determined the changes in the content of norepinephrine, epinephrine, dopamine and its metabolite dihydroxy phenyl acetic acid (DOPAC) in the right atria during morphine tolerance and withdrawal. In addition, we examined if such changes were correlated with alterations in atrial rate in isolated right atria of the rat. As an index of HPA activity, simultaneously with atrial content of catecholamines, we measured plasma concentrations of corticosterone.

Materials and methods

The study was approved by the local Animal Use Committee. Male Sprague–Dawley rats (200–250 g) were housed 4–5 per cage under a 12-h light–dark cycle (light 08:00–20:00) in a room with controlled temperature (22 ± 1 °C) and humidity (50 ± 10%), and food and water were available ad libitum.

EXPERIMENTAL PROCEDURE

On the basis of previous studies, rats were rendered dependent on morphine by s.c. implantation of morphine base pellets (75 mg). The implantation schedule consisted of one pellet on day 0, two pellets on day 2 and three pellets on day 4. Control animals received placebo pellets (lactose) according to the same schedule. A withdrawal syndrome was induced by injecting naloxone 1 mg kg⁻¹ s.c. on the morning of sacrifice (day 7) to rats implanted with morphine pellets. The control group implanted with placebo pellets was also injected with naloxone. Animals were killed 30 min after injection.

There were four experimental groups: (1) chronic placebo–acute saline s.c.; (2) chronic placebo–acute naloxone s.c.; (3) chronic morphine–acute saline s.c.; and (4) chronic morphine–acute naloxone s.c.

CORTICOSTERONE ASSAYS

At the end of treatment, rats were killed by decapitation between 09:30–10:00 to avoid circadian variations in plasma concentrations of corticosterone. Trunk blood was collected into ice-cooled tubes containing 5% EDTA and centrifuged (2500 rpm, 4 °C, 15 min). Plasma was separated and stored at −30 °C until assayed for corticosterone. Plasma concentrations of corticosterone were measured using a commercially available kit for rats (¹²⁵I-corticosterone radioimmunoassay, ICN, Biomedicals, USA). 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.

ANALYTICAL PROCEDURE FOR MEASUREMENT OF ATRIAL CATECHOLAMINES

Norepinephrine, epinephrine, dopamine and DOPAC were estimated in the right atrium of the rat by high pressure liquid chromatography with electrochemical detection (HPLC/ED, Waters Millipore, MA, USA). After decapitation, the chest was opened with a midsternal incision and the right atrium dissected and stored at −80 °C until assayed for catecholamines. The right atrium was weighed and placed immediately in a dry ice-cooled polypropylene vial and homogenized with a Polytron homogenizer (setting 5 for 30 s) in 1 ml of perchloric acid 0.1 mol litre⁻¹ containing EDTA 2.7 mmol litre⁻¹ and 3,4 dihydroxy-benzylamine (DHBA) 5 pg µl⁻¹ (Waters, MA, USA) as internal standard. The homogenates were centrifuged (15 000 rpm, 4 °C, 15 min), the supernatant layer removed into a 1-ml syringe and filtered through a 0.22-µm GV (Millipore), and 10 µl of each sample injected into a 5-µm C18 reserve-phase column (Waters). Electrochemical detection was accomplished with a glassy carbon electrode set at a potential of +0.65 V versus the silver–silver chloride reference electrode. The mobile phase consisted of a 95:5 (v/v) mixture of water and methanol with sodium acetate 50 mmol litre⁻¹, citric acid 20 mmol litre⁻¹, 1-octyl-sodium sulphonate 3.75 mmol litre⁻¹, 9, di-n-butylamine 1 mmol litre⁻¹ and EDTA 0.135 mmol litre⁻¹, adjusted to pH 4.3. Flow rate was 0.9 ml min⁻¹. Chromatographic data were analysed with a Waters 740 date module integrator and quantified using the peak area ratio of the internal standard. Atrial content of catecholamines was expressed as ng g⁻¹ wet weight of tissue.

ISOLATED RIGHT ATRIA PREPARATION

The right atrium was isolated and suspended in a 10-ml organ bath. Tyrode solution of the following composition (mmol litre⁻¹) was used: NaCl 136.9; KCl 5.0; MgCl₂ 1.05; CaCl₂ 1.8; NaH₂PO₄ 0.4; NaHCO₃ 11.9; glucose 0.5. The bathing solution was maintained at 37 °C, pH 7.4 and bubbled with 95% oxygen and 5% carbon dioxide. Each preparation was suspended under a resting tension of 0.5 g and equilibrated for 45 min before the start of the experiment. The right atrium was being spontaneously and the frequency of contraction was measured using a force displacement transducer (Grass FT-03) and recorded on a Letica polygraph. Only preparations that had a stable basal frequency of contraction at the end of the stabilization period were accepted for study.

INDUCTION OF PHYSICAL DEPENDENCE IN VITRO

Concentration–response curves were constructed for naloxone 10⁻³–10⁻⁵ mol litre⁻¹ from the right atria in rats treated chronically with morphine or placebo pellets. Concentration–response curves for d-naloxone (inactive isomer) were also constructed in both types of preparations. Concentrations of drugs were increased after a steady-state response had been attained with the previous concentration.

DRUGS

Pellets of morphine base (Alcaliber Labs, Madrid, Spain) or lactose were prepared by the Department of Pharmacy, Clinic Hospital (Madrid, Spain). Naloxone HCl (Merck, Sharp and Dome, Madrid, Spain) and d-naloxone HCl (Endo Inc. New York,
Acute administration of naloxone 1 mg kg\(^{-1}\) to morphine-treated animals decreased \((P<0.001)\) atrial content of norepinephrine \((290.2 (11.9) \text{ ng g}^{-1})\), epinephrine \((15.6 (2.1) \text{ ng g}^{-1})\) and dopamine \((9.52 (0.5) \text{ ng g}^{-1})\), whereas the DOPAC/DA ratio \((1.38 (0.2))\) increased \((P<0.001)\) compared with the saline-injected group (norepinephrine 783.4 (52.2) ng g\(^{-1}\); epinephrine 48.2 (3.6) ng g\(^{-1}\); dopamine 37.2 (4.5) ng g\(^{-1}\); and DOPAC/DA ratio 0.34 (0.04)).

However, administration of naloxone to placebo-treated animals did not modify concentrations of norepinephrine \((601.0 (27.1) \text{ ng g}^{-1})\), dopamine \((27.6 (1.1) \text{ ng g}^{-1})\), DOPAC \((24.9 (2.4) \text{ ng g}^{-1})\) or the DOPAC/DA ratio \((0.9 (0.08))\) but significantly \((P<0.001)\) increased atrial content of epinephrine \((35.6 (2.0) \text{ ng g}^{-1})\) compared with the respective control group injected with saline (figs 1, 2).

Plasma concentrations of corticosterone increased \((P<0.001)\) 30 min after naloxone injection in morphine-treated rats \((435.8 (27.6) \text{ mg ml}^{-1})\) compared with morphine-treated rats injected with saline \((142.0 (17.5) \text{ ng ml}^{-1})\) or placebo-treated rats injected with naloxone \((166.5 (13.3) \text{ mg ml}^{-1})\) (fig. 3). Plasma concentrations of corticosterone in placebo-treated rats injected with naloxone were similar to those in the respective control group given saline \((168.5 (8.6) \text{ mg ml}^{-1})\).

**EFFECTS OF NALOXONE ON ATRIAL RATE IN THE ISOLATED RIGHT ATRIA FROM CHRONICALLY PLACEBO- OR MORPHINE-TREATED RATS**

In placebo-treated rats, naloxone \(10^{-11}-10^{-3} \text{ mol litre}^{-1}\) decreased the frequency of contraction in a concentration-dependent manner. The maximum effect was obtained at \(10^{-4} \text{ mol litre}^{-1}\) \((24 (0.9)\%\). In contrast, in morphine-treated rats, naloxone increased atrial rate in a concentration-dependent manner, producing a maximum effect of \(30 (0.1)\%\) at \(10^{-4} \text{ mol litre}^{-1}\) (fig. 4a). The inactive isomer \(\alpha\)-naloxone induced similar effects in placebo and morphine-treated rats. Thus \(\alpha\)-naloxone produced a decrease in atrial rate at concentrations of \(10^{-11}-10^{-3} \text{ mol litre}^{-1}\) in placebo- or morphine-treated rats. In both groups the maximum effect was \(30 (1.2)\%\) (fig. 4a).

**Discussion**

Different methods have been used in several studies to induce opioid tolerance–dependence. In the present study, the method of morphine pellet implantation and the schedule used were similar to those described previously,\(^{16-19}\) which produce a high degree of tolerance to the effects of morphine in the central nervous system. Moreover, opioid tolerance and withdrawal syndrome can be demonstrated in isolated tissues, such as in myenteric plexus longitudinal muscle (MPLM) strips from guineapig.\(^{17,19}\) However, few studies have been performed in cardiac tissues.\(^{15,20}\) Hence the mechanisms involved in opioid dependence in the heart are poorly understood.

In the present study the concentration–response curves to naloxone on isolated right atria were constructed approximately 1 h 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 dependence have been observed in preparations that were maintained in morphine or examined in the absence of the opioid.\(^{21}\) However, experiments in tissues that were bathed in a solution...
containing morphine were difficult to quantify and interpret as the magnitude of the inhibitory effects produced by the maintenance concentration were unknown and therefore the degree of tolerance was also imprecise.22

In our study administration of naloxone to morphine-treated rats caused behavioural signs and symptoms of opioid withdrawal, such as wet-dog shakes, diarrhoea, escape jumps, teeth chattering, salivation, ptosis and irritability (data not shown). Moreover, our data showed that in morphine-treated rats, acute administration of naloxone decreased atrial content of norepinephrine, epinephrine and dopamine, whereas the ratio DOPAC/DA (an index of dopamine turnover) was increased. As expected, a large corticosterone response was observed after naloxone-induced withdrawal.

The mechanical response to naloxone in isolated right atria was different in preparations from naïve and tolerant rats. Thus in preparations from morphine-treated rats, naloxone induced a dose-dependent increase in heart rate whereas in preparations from placebo-treated rats, naloxone decreased this variable. In contrast, D-naloxone decreased the frequency of contraction in preparations from placebo- and morphine-treated rats. As D-naloxone is an inactive isomer, the decrease in the frequency of contraction obtained in our study was unlikely to be mediated via the opioid receptor. There is clear evidence that one of the autonomic symptoms of the withdrawal syndrome is tachycardia. This response could result from release of catecholamines in cardiac tissue. In addition, our results showed that chronic morphine treatment produced neuroendocrine physical dependence, as evidenced by an increase in corticosterone secretion 30 min after naloxone injection.

Several studies of opioid dependence in the central nervous system demonstrated that precipitation of withdrawal by administration of opioid antagonists to morphine-dependent rats caused an increase in firing of norepinephrine neurones in the locus coeruleus and an increase in the turnover of norepinephrine in the forebrain, in addition to a behavioural syndrome that has been associated with these effects.5 6 Many symptoms of the autonomic nervous system which may be caused both by increasing activity of the locus coeruleus and by increased activity of spinal centres controlling sympathetic outflow.23 In addition, chronic administration of opioids has been shown to increase concentrations of cAMP-dependent protein kinase and substrates for protein kinase, including tyrosine hydroxylase24 (the rate-limiting enzyme in the biosynthesis of catecholamines) in the locus coeruleus. Moreover, studies in our laboratory have demonstrated that naloxone administration to tolerant rats enhanced plasma concentrations of corticosterone simultaneously with a reduction in hypothalamic content of norepinephrine.11 12 As the paraventricular nucleus is a major area governing the output of the autonomic nervous system and ACTH secretion, the increase in corticosterone production and changes in heart catecholaminergic activity observed in our study can be attributed to a blunted response in the paraventricular nucleus.

Despite substantial evidence that noradrenergic neurones in the locus coeruleus and their projection to the hypothalamus are involved in opioid dependence and withdrawal,41 42 there are few reports on the characteristics of functional disturbances of heart catecholaminergic neurones during opioid tolerance and dependence. Previous studies14 15 24 have demon-

**Figure 2** Atrial dopamine (A) and DOPAC (B) concentrations, and DOPAC/DA ratio (C) in placebo- and morphine-pelleted rats, 30 min after acute injection of saline s.c. or naloxone 1 mg kg⁻¹ s.c. (mean (SEM) of 4–5 experiments). ***P<0.001 compared with morphine with saline; †P<0.05, ††P<0.01, †††P<0.001 compared with placebo with naloxone; ‡‡P<0.01 compared with placebo with saline.

**Figure 3** Plasma concentrations of corticosterone in placebo- and morphine-pelleted rats, 30 min after acute injection of saline s.c. or naloxone 1 mg kg⁻¹ s.c. (mean (SEM) of 6–8 experiments). ***P<0.001 compared with morphine with saline; †††P<0.001 compared with placebo with naloxone.
stratified that administration of naloxone in rats pretreated with a single dose of morphine, infusions of morphine or by chronic treatment with morphine pellets precipitated a withdrawal response, including an increase in mean arterial pressure, biphasic heart rate response and an increase in plasma concentrations of norepinephrine and epinephrine, suggesting that cardiovascular abstinence is mediated mostly by the catecholaminergic systems. Our results demonstrated clearly that catecholamines play an important role in the sympathetic manifestations of abstinence syndrome. Naloxone-induced activation of pithalamic–pituitary–adrenocortical and cardiovascular system in the rat. Neuroneuroendocrinology 1989; 49: 181–190.


