Catecholaminergic activity and 3′,5′-cyclic adenosine monophosphate concentrations in the right ventricle after acute and chronic morphine administration in the rat

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We have examined possible regulation of norepinephrine and dopamine concentrations and turnover in the right ventricle of the rat after acute administration of saline i.p. or morphine 30 mg kg⁻¹ i.p. to placebo (naïve) or morphine (tolerant) pretreated rats. We also assessed concentrations of 3′,5′-cyclic adenosine monophosphate (cAMP) in the right ventricle after the same treatments. Concentrations of catecholamines and their metabolites in the heart were measured by high-pressure liquid chromatography with electrochemical detection (HPLC/DE). Concentrations of cAMP in the heart were measured by radioimmunoassay (RIA). Administration of morphine to naïve rats did not modify concentrations of norepinephrine (NE), normetanephrine (NMN) or NMN/NE ratio in the right ventricle. However, dopamine concentrations increased whereas dopamine turnover decreased. In addition, cAMP concentrations decreased after acute administration of morphine to naïve rats. In rats pretreated with morphine chronically, there was an increase in norepinephrine concentrations with no change in normetanephrine concentrations or norepinephrine turnover after acute injection of morphine. In contrast, dopamine turnover increased in the tolerant groups after acute injection of saline or morphine compared with the naïve group given morphine, indicating that tolerance develops to the acute effects of the opioid. Concentrations of cAMP increased after chronic morphine administration. Our results demonstrate that chronic morphine pretreatment leads to up-regulation of the cAMP system in the heart and suggest that this up-regulation may be involved in the cellular mechanisms implicated in the adaptive changes of dopaminergic neurones in the heart observed during chronic treatment with morphine.

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Opioid peptides have been shown to have a wide tissue distribution and are known to control cardiac function via reflex mechanisms involving the central nervous system or modulation of neurotransmitter release from neurones located within the heart.1 The discovery that mammalian myocardial cells have opioid receptors2–6 led to studies aimed at investigating direct myocardial effects caused by opioid receptor stimulation and identifying possible intracellular opioid pathways.

Repeated use of opioids induces adaptive changes in the central and peripheral nervous system, leading to development of tolerance and dependence. Several animal models have been used to investigate the mechanisms involved in the response to opioids and in the development of tolerance and dependence.7 Many compensatory mechanisms have been described in the central nervous system, including up-regulation of the 3′,5′-cyclic adenosine monophosphate (cAMP) system and alteration of gene expression.8,9 Catecholaminergic systems play an important role in the development and maintenance of opioid tolerance–dependence and in the effects on drug withdrawal, as has been shown in several neuronal models such as the locus coeruleus and nucleus accumbens.7,10 Despite substantial evidence that catecholaminergic neurones in the locus coeruleus and projections to the hypothalamus are involved in opioid tolerance–depend-
Catecholamines and cAMP content in the heart after chronic morphine administration

There are few data on alterations of heart catecholaminergic neurones during morphine tolerance and 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. In addition, previous studies in our laboratory demonstrated that acute administration of morphine in morphine-treated rats produced a decrease in the turnover of dopamine in auricular tissues which could be responsible for the decrease in force and frequency of contraction observed in in vitro experiments.

As tolerance may include the central and peripheral nervous system, the aim of our study was to elucidate the role of norepinephrine and dopamine systems in the cardiac response to acute morphine injection and the adaptive changes on chronic morphine administration. Thus we measured norepinephrine and dopamine content and turnover in the right ventricle of the rat after chronic morphine administration. We also examined modulation of cAMP formation in the right ventricle in response to morphine treatment.

Materials and methods

The study was approved by the Local Animal Use Committee. Male Sprague–Dawley rats (200–300 g at the beginning of treatment) were housed 4–5 per cage under a 12-h light–dark cycle in a room with controlled temperature (22±11°C), humidity (50±10%) and food and water ad libitum.

Experimental procedure

On the basis of previous studies, rats were rendered tolerant to morphine by subcutaneous 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 treatment results in a profound state of tolerance–dependence. Naïve animals were implanted with placebo pellets containing lactose at the same time. On day 7, animals were treated acutely with saline i.p. or morphine 30 mg kg⁻¹ i.p. and killed 30 min later. There were four experimental groups for measurement of catecholamines in the right ventricle: (1) chronic placebo (naïve)–acute saline i.p.; (2) chronic placebo (naïve)–acute morphine i.p.; (3) chronic morphine (tolerant)–acute saline i.p.; and (4) chronic morphine (tolerant)–acute morphine i.p. Another four groups, who received the same treatments, were used for cAMP analysis in the right ventricle. Weight gain of the rats was checked to ensure that morphine was liberated correctly from the pellets as it is known that chronic morphine treatment induces a decrease in body weight gain because of lower caloric intake.

In addition, on the day of the experiment, body weight was measured immediately before injection of saline or morphine and 30 min later, immediately before killing.

Estimation of catecholamines and their metabolites in the right ventricle

After decapitation, the chest was opened using a mid-sternal incision and the right ventricle was dissected and stored immediately at –80°C. Norepinephrine and its metabolite normetanephrine, and dopamine and its metabolite 3,4-dihydroxyphenyl acetic acid (DOPAC) were measured by high-pressure 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 4 for 40 s) in perchloric acid 1.5 ml (0.1 mol litre⁻¹). The homogenates were centrifuged (20 000 rpm, 4°C, 15 min), the supernatant layer 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). Each sample (10 µl) was injected into a 5-µm C₁₈ reverse-phase column (Waters) 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 Ag–AgCl reference electrode (Waters). The mobile phase consisted of a 95:5 (v/v) mixture of water and methanol with sodium acetate 20 mmol litre⁻¹, citric acid 50 mmol litre⁻¹, 1-octyl-sodium sulphonate 3.75 mmol litre⁻¹, 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 and chromatographic data were analysed using Millennium 2010 Chromatography Manager (Millipore) equipment.

DOPAC, norepinephrine, normetanephrine and dopamine were simultaneously detected by the HPLC method at elution times of 3.12, 4.00, 7.00 and 10.70 min, respectively. Norepinephrine, dopamine and their respective metabolites were quantified by reference to calibration curves run at the beginning and end of each series of assays. Linear relationships were observed between the amount of standard injected and measured peak heights. The content of norepinephrine, dopamine, normetanephrine and DOPAC in the right ventricle was expressed as ng g⁻¹ wet weight of tissue.

Measurement of cAMP in the right ventricle

Concentrations of cAMP were measured by radioimmunoassay (¹²⁵I-TME-S-cAMP, Diagnostic Pasteur, France) according to the manufacturer’s instructions. After dissection, the right ventricle was weighed and homogenized in cold perchloric acid 1.5 ml (0.3 mol litre⁻¹) with a Polytron homogenizer and centrifuged (12 000 rpm, 4°C, 15 min). The supernatants were treated with potassium phosphate until pH 6.2 was reached. The sensitivity of the assay was 2 pmol ml⁻¹. Intra- and inter-assay coefficients of variation were 7.7% and 8.2%, respectively. The antibody cross-reacted 100% with 3',5'-cAMP and less than 0.3% with other nucleotides. cAMP concentrations were expressed as nmol g⁻¹ of tissue.
**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). Norepinephrine bitartrate, normetanephrine, dopamine HCl and DOPAC (used as HPLC standards) were purchased from Sigma Chemical Co. (St Louis, MO, USA). Morphine HCl (Alcaliber Labs., Madrid, Spain) was prepared fresh daily, dissolved in sterile 0.9% NaCl (saline) and injected in volumes of 0.15 ml/100 g body weight. Other reagents were of analytical grade.

**Statistical analysis**

Data are expressed as mean (SEM). The significance of differences in content of norepinephrine (NE), normetanephrine (NMN), dopamine (DA) and DOPAC, and in NMN/NE and DOPAC/DA ratios were determined by analysis of variance followed by the Newman–Keuls test, using a computer program. The non-paired Student’s t test was used when comparing mean changes in body weight. Significance was taken as \( P<0.05 \).

**Results**

Rats rendered tolerant to morphine showed a significantly lower gain in body weight on day 7 than groups treated with placebo pellets. In addition, 7 days after the beginning of implantation of the morphine pellets, rats did not show any of the behavioural or physical signs of opioid withdrawal (data not shown).

**Norepinephrine concentrations and turnover after acute or chronic morphine administration**

Acute administration of morphine 30 mg kg\(^{-1}\) i.p. to naive rats did not alter normetanephrine concentrations or norepinephrine turnover (as estimated using the NMN/NE ratio) (Fig. 1). Figure 1 also shows norepinephrine content and turnover in rats rendered tolerant to morphine. The morphine-pretreated group injected acutely with saline on day 7 showed no changes in norepinephrine or normetanephrine concentrations or norepinephrine turnover compared with the corresponding naive group. In rats rendered tolerant to the opioid, concentrations of norepinephrine increased significantly 30 min after injection of morphine, with no changes in normetanephrine content or norepinephrine turnover.

**Dopamine concentrations and turnover after acute and chronic morphine administration**

Acute administration of morphine 30 mg kg\(^{-1}\) i.p. to naive rats increased significantly concentrations of dopamine, whereas dopamine turnover (as estimated using the DOPAC/DA ratio) decreased. The morphine-pretreated group injected acutely with saline showed no changes in dopamine concentrations whereas DOPAC concentrations increased compared with the corresponding naive group. Dopamine turnover increased in the tolerant group injected with saline compared with the naive group injected with morphine, indicating that tolerance develops to the decreased dopamine turnover by morphine (Fig. 2). In the morphine-pretreated rats, acute injection of morphine increased DOPAC concentrations and dopamine turnover compared with the naive group injected with morphine. In addition, there were significant differences in the concentrations of dopamine and DOPAC between the two morphine-pretreated groups injected with saline i.p. or morphine i.p.

**cAMP concentrations after acute or chronic morphine administration**

Concentrations of cAMP in the right ventricle obtained from rats implanted with placebo or morphine pellets are shown in Figure 3. Morphine 30 mg kg\(^{-1}\) given to placebo pretreated rats produced a decrease in cAMP concentrations compared with the naive group injected with saline. In morphine-tolerant rats, cAMP concentrations 30 min after saline i.p. or morphine i.p. were significantly higher than those obtained in their respective control groups.
Catecholamines and cAMP content in the heart after chronic morphine administration

Fig 2 Ventricular dopamine (DA), 3,4 dihydroxy acetic acid (DOPAC) and DOPAC/DA ratio in placebo (naive) and morphine-pretreated (tolerant) rats, 30 min after acute injection of saline i.p. or morphine 30 mg kg⁻¹ i.p. Values are mean (SEM); n=5–6 per group. ‡‡‡P<0.001 vs placebo + saline; ***P<0.001 vs placebo + morphine.

Fig 3 Ventricular cAMP in placebo (naive) and morphine-pretreated (tolerant) rats, 30 min after acute injection of saline i.p. or morphine 30 mg kg⁻¹ i.p. Values are mean (SEM); n=5–7 per group. ‡P<0.05, ‡‡P<0.01 vs placebo + saline; ***P<0.001 vs placebo + morphine.

existence of a dopaminergic system in the heart. We found that norepinephrine turnover in the ventricle after chronic morphine administration was similar to that found after acute injection of the opioid. However, chronic morphine exposure evoked a decrease in dopamine turnover, indicating that tolerance develops to the effects of morphine on the dopamine system in the right ventricle. In contrast, previous studies in our laboratory demonstrated that administration of morphine to morphine-pretreated rats caused a decrease in dopamine turnover in auricular tissues.13 14 These differences suggest that chronic morphine treatment induces different adaptive changes in the heart depending on the tissue examined (atria or ventricle).

Fig 2 Ventricular dopamine (DA), 3,4 dihydroxy acetic acid (DOPAC) and DOPAC/DA ratio in placebo (naive) and morphine-pretreated (tolerant) rats, 30 min after acute injection of saline i.p. or morphine 30 mg kg⁻¹ i.p. Values are mean (SEM); n=5–6 per group. ‡‡‡P<0.001 vs placebo + saline; ***P<0.001 vs placebo + morphine.

Discussion

The mechanisms underlying the actions of chronic opioid administration and the manner in which responses to morphine are co-ordinated in cardiac tissue are not well understood. We have investigated the adaptive changes in heart norepinephrine and dopamine neurones and concomitant opioid modulation of cAMP formation on exposure to the preferential µ agonist morphine.

Our results showed that acute injection of morphine to placebo-pretreated rats did not produce changes in norepinephrine turnover, whereas DOPAC/DA ratio (an index of dopamine turnover) decreased. These data indicate that the effects of morphine in the ventricle were associated with a decrease in the activity of dopaminergic neurones. The possible distribution of dopaminergic inputs through the heart is not known, although previous studies found detectable concentrations of dopamine in the heart.21 22 In addition, it has been demonstrated that D1 and D2 receptor genes were expressed in the atria and ventricle of rat heart.23 24 Hence, these findings and our results suggest the

Fig 3 Ventricular cAMP in placebo (naive) and morphine-pretreated (tolerant) rats, 30 min after acute injection of saline i.p. or morphine 30 mg kg⁻¹ i.p. Values are mean (SEM); n=5–7 per group. ‡P<0.05, ‡‡P<0.01 vs placebo + saline; ***P<0.001 vs placebo + morphine.

It has been demonstrated previously that development of tolerance to the mechanical effects of U-50,488H in the heart after chronic treatment with the agonist was not accompanied by down-regulation of κ binding sites,12 suggesting that tolerance in the heart may be associated with a functional change in heart neurones, unrelated to the receptor system. Collectively, these findings and our results support the hypothesis that dopaminergic neurones may be implicated in the adaptive changes observed in the heart after chronic exposure to the drug.

We found that acute treatment with morphine to placebo-pretreated rats produced a decrease in cAMP concentrations in the right ventricle. It is believed widely that acute activation of opioid receptors involves inhibition of neuronal activity. This inhibition is caused by co-ordinated changes at the cellular level including, possibly, inhibition of cAMP formation.25 Thus previous studies in neuronal cells have indicated that all three subtypes of opioid receptor inhibit adenylate cyclase in a pertussis toxin-sensitive manner.26–29 Also, in neurones of cardiac tissues, stimulation of opioid receptors reduces cAMP concentrations.30 31 Our results are in agreement with studies showing that cAMP concentrations in the ventricle decrease after acute morphine administration.

Whereas acute opioid administration inhibits adenylate cyclase activity, chronic opioid treatment leads to up-regulation of the cAMP system in different brain regions.8 32 33

Despite substantial evidence that the cAMP system in the central nervous system is involved in opioid tolerance,
there are no data on the characteristics of the functional disturbances of the heart cAMP system after chronic administration of morphine. Our results demonstrated clearly that chronic treatment with morphine produced an increase in ventricular concentrations of cAMP and suggest that up-regulation of cAMP may contribute to expression of tolerance to the cardiac effects of morphine.

In summary, we have demonstrated that acute administration of morphine produced a decrease in dopamine turnover in the heart with a compensatory change (increase in dopamine turnover) in dopaminergic neurones as a result of chronic morphine treatment. Moreover, we have shown for the first time that chronic morphine administration caused up-regulation of the cAMP system in the heart and suggest that this up-regulation may be involved in the cellular mechanism implicated in the adaptive changes of the heart dopaminergic neurones during chronic treatment with morphine. These data may be important in understanding the changes induced in the heart in subjects dependent on opioids who receive opioid agonists.

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