Effects of morphine on atrial preparations obtained from non-failing and failing human hearts

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Summary
We have examined the effects of morphine in auricular myocardium from non-failing and failing human hearts. In both preparations morphine induced inhibition. These responses were not antagonized by naloxone. Comparison of mean IC₃₀ values obtained in non-failing (3.9 (SEM 0.2) × 10⁻⁸ mol litre⁻¹) and failing (5010 (200) × 10⁻⁸ mol litre⁻¹) hearts indicates that the morphine concentration–response curves were significantly (P < 0.001) shifted to the right in preparations from failing hearts. In addition, a decrease in the maximal response was observed. These data indicate that opioid receptors are not involved in the cardiac effects induced by morphine and that there is a decrease in responses to morphine in the failing heart. (Br. J. Anaesth. 1996; 76: 106–110)

Key words

It has been demonstrated that morphine induces negative inotropic and chronotropic effects on isolated left and right atria of the rat [1–3]. However, it has also been shown in different heart muscle preparations from mammalian species, including humans, that morphine fails to influence cardiac function directly [4]. Despite numerous studies, the possible mechanism involved in the cardiac inhibitory effects of this drug are controversial. Recently, it has been postulated that opioid agonists have complex cardiovascular actions. Some effects may be mediated via opioid receptors while others are independent of opioid receptors [5].

On the other hand it is known that the opioid system is involved in various stress situations such as shock [6] and heart failure [7]. Thus in acute heart failure patients, the β-endorphin, met-enkephalin, and dynorphin plasma concentrations are increased, which provides evidence of activation of the opioid system [8]. However, in patients with chronic heart failure, plasma concentrations of β-endorphin and lipotrophin are decreased, which is interpreted as exhaustion of the opioid system [9].

Despite the well known fact that opioids play a significant role in heart failure, there is little information on the cardiac action of morphine on the human heart, and no information on its effect in the failing human myocardium. Heart failure results in increased sympathetic drive with increased plasma noradrenaline concentrations [10]. Prolonged exposure of a cell to a drug usually results in reduction or down-regulation of functional cell surface receptor molecules. The mechanisms involved in receptor desensitization have been studied most extensively for the β adrenergic agonists. Thus we have investigated the effects of morphine in isolated human right atrium from failing and non-failing human hearts and any possible involvement of opioid receptors by undertaking similar experiments in the presence of naloxone.

Materials and methods

Patients
The study was approved by the Institutional Research Practices Committee. All patients gave written informed consent. The Committee of Human research also approved the study.

Human right atrial appendages were obtained from 20 patients (12 male) without apparent heart failure (mean age 50.1 (range 36–58) yr) with mitral valve disease (NYHA classes I–II). Myocardium from failing human hearts was obtained from 20 patients (14 male) (mean age 52.8 (range 40–69) yr) with severe mitral valve disease (NYHA classes III–IV). In all patients auricular muscle was obtained from tissue resected during cardiac surgery before heart cannulation preceding extracorporeal circulation. None of these patients had been treated with sympathomimetic drug or β adrenergic receptor antagonists for at least 3 weeks before operation. Some patients were treated with nitrates (15), calcium antagonists (10) or diuretics (15), and occasionally with angiotensin-converting enzyme (ACE) inhibitors (n = 8) or digitalis glycosides (n = 3), alone or in combination.

General anaesthesia comprised fentanyl with benzodiazepines and isoflurane. Pancuronium was used as a neuromuscular blocker (the washout procedure described below was designed to eliminate any potential influence of these agents).

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Immediately after excision, the atrial muscle strips were placed in ice-cold pre-aerated Tyrode solution (for composition see below) and delivered to the laboratory within 15 min.

**PREPARATION OF TISSUES AND ORGAN BATH EXPERIMENTS**

The right atrial appendages were dissected to yield trabecula strips (4–5 mm in length and 1 mm or less in diameter) without endocardial damage and with fibres running parallel to the length of the strip.

The preparations were mounted in 10-ml organ baths at 37°C containing a modified Tyrode’s solution of the following composition (mmol litre⁻¹): NaCl 119.8; CaCl₂ 1.8; KCl 5.4; MgCl₂ 1.05; NaH₂PO₄ 0.42; NaHCO₃ 22.6; glucose 5.05; ascorbic acid 0.28; and disodium EDTA 0.05. The bathing solution was aerated with 95% oxygen and 5% carbon dioxide. Myocardial strips were stimulated electrically (Grass SD-9) by two platinum ring electrodes at a frequency of 1 Hz by rectangular pulses of 10 ms and supramaximal voltage (30 V). Each preparation was suspended under a resting tension of 0.5 g. After suspension in the organ baths, the tissues were washed four times every 10 min. Thereafter the preparations were allowed to equilibrate for 90 min and the bathing solution was changed once after 45 min. Isometric force of contraction was measured using a force displacement transducer (Grass FT-03) and recorded on a Dynograph Beckman polygraph. Each muscle preparation was used for one concentration–response curve.

Increased concentrations of morphine were added cumulatively to the organ bath at concentrations ranging from 10⁻¹⁰ to 10⁻³ mol litre⁻¹. Each drug concentration was added to the organ bath in a volume of 0.1 ml at 5-min intervals. Concentration–response curves to morphine were obtained also in the presence of naloxone (10⁻⁸, 10⁻⁷ or 10⁻⁶ mol litre⁻¹) added 15 min before morphine. Concentration–response curves were also constructed with naloxone 10⁻¹¹–10⁻⁴ mol litre⁻¹ (data not shown). In the time-control groups, equal volumes of distilled water (diluent for morphine and naloxone) were added to the tissue baths at the same times as those for the morphine and morphine with naloxone groups. To evaluate heart failure, we also constructed concentration–response curves with isoproterenol 10⁻¹¹–10⁻⁴ mol litre⁻¹ in preparations from non-failing and failing hearts.

The effects of morphine alone and isoproterenol are expressed as percentage change from baseline values. Similarly, the effects of morphine in the presence of naloxone are expressed as percentage change from baseline values after opioid antagonists.

**STATISTICS**

The results are expressed as means (SEM) of n experiments. Statistical comparisons were performed with one-way analysis of variance, followed by the Newman–Keuls post hoc test. Differences with P values, less than 0.05 were considered significant. IC₅₀, IC₅₀ and the slope were calculated by linear regression of the log concentration plot of the data with the aid of a computer program [12]. Mean slope values were compared using Student’s t test.

**COMPOUNDS**

The drugs used were morphine (Alcaliber Spain), naloxone hydrochloride (Merck, Sharp & Dohme, Spain) and isoproterenol hydrochloride (Sigma, Spain).

**Results**

The maximal effect on force of contraction occurred within 3–4 min after adding morphine or isoproterenol to the bath. All concentrations of isoproterenol tested significantly increased the force of contraction in a dose-related pattern compared with control. The maximal effect obtained in the non-failing heart (97.2 (3.2) %) was significantly (P < 0.01) higher than that obtained in the failing heart (51.2 (1.1) %). Similarly, the IC₅₀ for isoproterenol was significantly (P < 0.001) lower in non-failing compared with failing hearts (9.3 (0.2) × 10⁻⁸ and 120 (5.8) × 10⁻⁶ mol litre⁻¹, respectively). Thus isoproterenol was more potent in non-failing than in failing hearts.

There were no significant differences between the time-matched control groups obtained in non-failing and failing hearts (fig. 1). In both cases, morphine induced cardiac inhibition. In the non-failing heart, morphine significantly reduced (P < 0.05; P < 0.01; P < 0.001) cardiac inhibition at concentrations ranging from 10⁻⁸ to 10⁻³ mol litre⁻¹ compared with
the time-matched control. However, the inhibition caused by morphine was reduced in failing compared with non-failing hearts (fig. 1). Thus the maximal effect obtained by morphine (32 (1.0)%) was lower (P < 0.001) than that obtained in the non-failing heart (68 (1.5)%). Comparison of IC₃₀ values (tables 1, 2) indicated that the morphine concentration–

**Figure 2** Concentration–response curves for morphine in isolated electrically stimulated human right atrial strips from non-failing hearts in the absence (○, n = 8) and presence of naloxone 10⁻⁵ (△, n = 4), 10⁻⁶ (□, n = 4) or 10⁻⁷ (□, n = 4) mol litre⁻¹. Average auricular inotropism (mean (SEM)) in the absence of naloxone was 0.99 (0.05) g and 0.93 (0.05) g, 0.96 (0.08) g and 0.98 (0.05) g in the presence of naloxone 10⁻⁵, 10⁻⁶ and 10⁻⁷ mol litre⁻¹, respectively. Each point represents mean (SEM). *P < 0.05; **P < 0.01; ***P < 0.001 compared with morphine alone.

**Table 1** Maximal inhibitory effects, IC₃₀ and slope for morphine in the absence or presence of naloxone (Nx) in auricular strips of patients without heart failure (mean (SEM)) (n = 4–8). Lower and upper = minimum and maximum slopes of the concentration–response curves

<table>
<thead>
<tr>
<th>Drugs</th>
<th>% Maximum inhibition</th>
<th>IC₃₀ x 10⁻⁸ mol litre⁻¹</th>
<th>Slope</th>
<th>r</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine</td>
<td>68 (1.5)</td>
<td>3.9 (0.2)</td>
<td>8.9 (0.7)</td>
<td>0.98</td>
<td>7.1</td>
<td>10.7</td>
</tr>
<tr>
<td>Morphine + Nx</td>
<td>69 (1.2)</td>
<td>4.8 (0.9)</td>
<td>9.2 (0.8)</td>
<td>0.97</td>
<td>7.2</td>
<td>11.2</td>
</tr>
<tr>
<td>Morphine + Nx</td>
<td>67 (1.4)</td>
<td>5.1 (0.8)</td>
<td>9.0 (0.7)</td>
<td>0.98</td>
<td>7.2</td>
<td>10.8</td>
</tr>
<tr>
<td>Morphine + Nx</td>
<td>68 (1.3)</td>
<td>5.6 (1.0)</td>
<td>9.1 (0.7)</td>
<td>0.97</td>
<td>7.2</td>
<td>11.1</td>
</tr>
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**Table 2** Maximal inhibitory effects, IC₃₀ and slope for morphine in the absence or presence of naloxone (Nx) in auricular strips of patients with heart failure (mean (SEM)) (n = 4–8). Lower and upper = minimum and maximum slopes of the concentration–response curves

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</tr>
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<tr>
<td>Morphine</td>
<td>32 (1.0)</td>
<td>5010 (200)</td>
<td>3.9 (0.2)</td>
<td>0.98</td>
<td>3.3</td>
<td>4.5</td>
</tr>
<tr>
<td>Morphine + Nx</td>
<td>33 (0.8)</td>
<td>5600 (250)</td>
<td>3.9 (0.2)</td>
<td>0.99</td>
<td>3.3</td>
<td>4.5</td>
</tr>
<tr>
<td>Morphine + Nx</td>
<td>33 (1.2)</td>
<td>6710 (220)</td>
<td>3.7 (0.2)</td>
<td>0.98</td>
<td>3.1</td>
<td>4.2</td>
</tr>
<tr>
<td>Morphine + Nx</td>
<td>34 (1.8)</td>
<td>6520 (190)</td>
<td>4.3 (0.2)</td>
<td>0.98</td>
<td>3.7</td>
<td>5.0</td>
</tr>
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</table>

**Discussion**

The high concentrations of morphine used in this study are equivalent to those in patients given morphine for pain relief. Thus high plasma morphine concentrations (> 10⁻⁸ mol litre⁻¹) occur during the first minute after morphine 10 mg i.v. [13]. It has been shown that morphine, DAMGO, DPDPE and U-50,488H induced cardiac inhibition in rats [1, 14]. Similarly, morphine inhibited auricular inotropism in a concentration-dependent manner in preparations from patients without heart failure. However, the cardiac inhibitory effects of morphine obtained in isolated left atria of the rat were lower than those observed in this study. These differences may represent an intrinsic difference in excitation–contraction coupling between human and rat myocardium.

The negative inotropic effects induced by morphine in both failing and non-failing hearts were not antagonized by naloxone. Although morphine has a higher selectivity for μ receptors, at the concentrations studied it can bind to σ and κ receptors, and...
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Therefore in this study we used high concentrations of naloxone to antagonize all three types of opioid receptor [15].

Our data demonstrated that opioid receptors were not involved in the inhibitory effects of morphine. Similarly, other authors [5,16–18] have described that the cardiac effects induced by high doses or concentrations of opioids are not mediated by opioid receptor activation. If the inhibitory cardiac response induced by morphine was not mediated by opioid receptors, then other mechanisms may be considered. One possibility is that the drug may be interacting with other receptor systems known to mediate inhibitory cardiac effects, such as the muscarinic receptor system; if so the muscarinic antagonist, atropine, should have eliminated this effects. However, previous studies in our laboratory have demonstrated that atropine failed to modify the negative inotropic and chronotropic effects induced by opioid agonists in isolated cardiac tissues [17,18], thus excluding the involvement of muscarinic receptors.

An earlier investigation [19] demonstrated an interaction between the opioid and adrenergic systems in spontaneously beating guineapig atria. Opioids inhibited the cardiac response to adrenergic stimulation in both atrial and ventricular preparations [20–22]. Therefore, another possible explanation for the inhibitory effects of opioids in these studies is presynaptic inhibition of the sympathetic nervous system. Thus a previous study in our laboratory showed that the negative inotropic effect induced by opioids in left atria of the rat was not apparent in the presence of reserpine [17].

Moreover, this response to opioid agonists was dependent on β adrenergic mechanisms as it was eliminated by prior block with propranolol [17].

Thus our data and the studies of others described above demonstrate that the negative inotropic action of morphine on the heart is mediated by a non-opioid mechanism and suggest that an adrenergic mechanism may be involved.

We have also demonstrated for the first time that the negative inotropic response to morphine was decreased markedly in heart failure. Thus the concentration–response curve for morphine was shifted to the right and the maximum inhibition to morphine was decreased in failing hearts. Changes in the concentration–response curves for opioid agonists, such as horizontal position, maximum effect and slope, may involve alterations in the affinity of the receptor for the agonist, the density of opioid receptors or the efficacy of the drug–receptor interaction [23]. However, our data clearly demonstrated that the inhibitory cardiac response to morphine was not mediated by opioid receptors, which leads to the conclusion that the decrease in negative inotropic response to opioids in failing hearts could not be explained by changes in the density or affinity of opioid receptors.

It is known that heart failure is characterized by profound β adrenergic receptor desensitization in the myocardium, apparent as depressed inotropic responsiveness to β adrenergic agonists [24,25], mediated by several disorders in β adrenergic receptor signalling [26,27]. Abnormal myocardial β adrenergic signal transduction is characterized by selective β1 receptor downregulation [11,28,29]. Our data are in agreement with these studies. Thus the positive inotropic effects induced by isoproterenol were decreased in the failing heart. Moreover, isoproterenol was more potent in the non-failing compared with the failing heart.

Another explanation for the inhibitory cardiac effects of opioids is interaction at the level of the post-receptor transduction cascades. It has been demonstrated that β adrenoceptor-sensitive adenylate cyclase is inhibited by µ opioid agonists in rat striatal neurons [30]. In addition, opioid agonists and antagonists potentiate the cardiac effect of β adrenergic agonists by a mechanism unrelated to the binding of these drugs to opioid receptors [31]. Moreover, propranolol antagonized the pressor effects of [met]enkephalin Arg6-Phen2 in the anaesthetized rat [32] and the excitatory effects of morphine on ventricular automaticity [33] while potentiating the negative chronotropic effects of U-50,488H [20]. In addition, naloxone potentiated the cardiovascular responses to sympathomimetic amines [34]. These studies support the concept that a β adrenergic mechanism could be involved in the cardiac effect of opioids. If an interaction between opioid agonists and β adrenergic receptors were responsible for the negative inotropic response to morphine obtained in our preparations, the decrease in the inhibitory cardiac response to morphine obtained in the failing heart could be explained by selective β1 adrenoceptor downregulation that occurs in heart failure.

Figure 3 Concentration–response curves for morphine in isolated electrically stimulated human right atrial strips from failing hearts in the absence (n = 8) and presence of naloxone 10⁻⁸ (□, n = 4), 10⁻⁷ (○, n = 4) or 10⁻⁶ (△, n = 4) mol litre⁻¹. Average auricular inotropism (mean (SEM)) in the absence of naloxone was 0.89 (0.02) g, and 0.91 (0.05) g, 0.99 (0.03) g and 0.99 (0.08) g in the presence of naloxone 10⁻⁸, 10⁻⁷ and 10⁻⁶ mol litre⁻¹, respectively. Each point represents mean (SEM). * P < 0.05; ** P < 0.01; *** P < 0.001 compared with morphine alone.
References


