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Short communication

The role of adenosine in the hypotensive actions of morphine

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Abstract

The role of adenosine in the hypotensive action of morphine was examined in the pentobarbitone anaesthetized or pithed rat preparations. Adenosine (10–300 μg/kg) induced dose-dependent decreases in diastolic blood pressure in the anaesthetized rat preparation. These effects were attenuated by infusion of 1,3-dipropyl-8-cyclopentylxanthine (DPCPX; 50 μg/kg/min), 8-phenyltheophylline (8PT; 50 μg/kg/min), and 3,7-dimethyl-1-propargylxanthine (DMPX; 50 μg/kg/min). In this preparation morphine (10–1000 μg/kg) also induced dose-dependent decreases in diastolic blood pressure. Guanethidine (16 μg/kg/min), atropine (1 mg/kg), DPCPX and 8PT reduced the effect of morphine on diastolic blood pressure, whilst DMPX (50 μg/kg/min) was inactive. In the pithed rat preparation morphine was inactive at doses up to 10 mg/kg. The results suggest that the hypotensive effect of morphine is mediated at least in part by the release of adenosine which then acts on centrally located adenosine receptors to induce changes in autonomic control of blood pressure.

Keywords: Morphine; Adenosine; Hypotension; Adenosine receptor

1. Introduction

Adenosine has often been implicated as a mediator of the effects of morphine, since methylxanthines, which are adenosine receptor antagonists, have been shown to block antinociception produced by intrathecal morphine administration (Sawynok et al., 1991) and to reduce morphine induced hypotension (Calignano et al., 1992). Since both adenosine and opiate receptors are linked to the adenylyl cyclase second messenger system, a potential for interaction may exist at this level.

Both morphine and endogenous opiate peptides such as β-endorphin have profound cardiovascular effects, including hypotension and bradycardia. Since opioid peptides have been found in brain regions concerned with cardiovascular control (Bolme et al., 1978; Holaday, 1983), it is possible that they may be involved in central regulation of blood pressure.

The present study was designed to examine the role of adenosine in the hypotensive effect of morphine using the anaesthetized rat preparation. Selective antagonists and adenosine uptake inhibitors were used to characterize the adenosine receptors involved in the cardiovascular actions of adenosine and morphine. Drugs which are confined to the peripheral circulation and pithed rat preparations were used to determine whether the effects of morphine occur at central or peripheral locations.

2. Materials and methods

2.1. Anaesthetized rat preparation

Female Hooded Wistar rats weighing 205–320 g were anaesthetized with pentobarbitone sodium (60 mg/kg i.p. initially, then 30 mg/kg i.v. subsequently if required). To maintain a patent airway, the trachea was isolated and cannulated using polyethylene (PE250) tubing. The left jugular vein, left femoral vein and right carotid artery were cannulated using polyethylene (PE50) tubing rinsed with either physiological saline (venous cannuli) or heparinised saline solution (100 IU heparin/ml saline, arterial cannula). Blood pressure was recorded at the carotid artery using a Gould Statham Physiological pressure transducer connected to a Grass 79D polygraph recorder. Following dissection the preparation was allowed 30 min to equilibrate.

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Adenosine (10–3000 μg/kg) and morphine (30 and 100 μg/kg) were administered via the jugular vein at 5 min intervals. The effects of adenosine receptor antagonists on responses to morphine were determined by repeating the dose-response curve to adenosine and the two responses to morphine in the presence of these drugs. Only one antagonist at one dose was used in each animal. The antagonists were 1,3-dipropyl-8-cyclopentylxanthine (DPCPX), 8-phenyltheophylline (8PT), 8-(*p*-sulfophenyl)theophylline (8SPT) and 3,7-dimethyl-1-propargylxanthine (DMPX). The antagonists were infused at 50 μg/kg/min via the femoral vein 15 min prior to the second dose-response curve, and maintained for the duration of the experiment. The effect of a combination of adenosine uptake inhibitors was also investigated. The combination consisted of lidoflazine (2.5 μg/kg/min), dipyridamole (0.5 μg/kg/min) and *S*-(4-nitrobenzyl)-6-thioinosine (NBTI, 0.82 μg/kg/min). The effects of guanethidine (16 μg/kg/min), naloxone (10 mg/kg bolus), mepyramine (10 μg/kg/min) and atropine (1 mg/kg bolus) on responses to morphine and adenosine were also determined. Prior to proceeding with the second dose-response curve in animals infused with guanethidine, a carotid artery occlusion test was performed to determine whether the hypertensive reflex response was still present. Control experiments were carried out using an infusion of the appropriate vehicle. Results were expressed as a percentage decrease of the resting diastolic blood pressure.

2.2. Pithed rat preparation

Rats were prepared as above prior to pithing. Pithing was performed according to the method described by Fozard et al. (1987). A metal rod was inserted through the orbit of the eye, and pushed through the brain and spinal cord. Once spontaneous breathing ceased the tracheal cannula was connected to a Parvalux respirator (63 breaths/min, 4.0 ml/breath). The animal was then given 0.1 mg/kg atropine to prevent accumulation of excess bronchial secretion. Since the pithing process resulted in a resting blood pressure of 45.5 ± 4.9 mm Hg ($n = 5$), phenylephrine infusion via the femoral vein (0.2 μg/kg/min) was used to elevate blood pressure of the rat to a level which allowed measurement of depressor responses (163.7 ± 11.7 mm Hg, compared with 112.6 ± 5.9 mm Hg for intact animals, $n = 10$). Dose-response curves to adenosine were performed as per intact anaesthetized rats, with the exception that drug doses were given at 3 min intervals in the pithed rats as the phenylephrine infusion was unable to maintain blood pressure for more than 2–20 min. Dose-response curves were separated by a 15 min antagonist equilibration period. Results were expressed as a percentage decrease of the phenylephrine-elevated blood pressure. The effects of morphine on blood pressure in the pithed rat were tested in two groups of rats, those with and those without phenylephrine infusion.

2.3. Materials

Drugs used included lidoflazine (Sigma), mepyramine maleate (Sigma), naloxone hydrochloride (Sigma), phenylephrine hydrochloride (Sigma), guanethidine sulphate (Sigma), atropine sulphate (Sigma), adenosine (Research Biochemicals), 8-cyclopentyl-1,3-dipropylxanthine (DCPCX, Research Biochemicals), 8-phenyltheophylline (8PT, Research Biochemicals), 8-(*p*-sulfophenyl)theophylline (8SPT, Research Biochemicals), 3,7-dimethyl-1-propargylxanthine (DMPX, Research Biochemicals), *S*-4-nitrobenzyl-6-thioinosine (NBTI, Research Biochemicals), morphine hydrochloride (Macfarlane Smith), and pentobarbitone sodium (Boehringer Ingelheim). All drugs except DPCPX, 8PT and the uptake inhibitor combination were dissolved in saline. DPCPX, 8PT and the uptake inhibitor combination (10 mM) were dissolved in dimethyl sulfoxide (DMSO) and diluted to the required concentration in saline, with a resulting solution of less than 10% DMSO. A stock solution of the uptake inhibitors was made weekly. The infused uptake inhibitor combination was prepared daily, to give final uptake inhibitor doses of 0.5 μg/kg/min dipyridamole, 0.82 μg/kg/min NBTI and 2.5 μg/kg/min lidoflazine.

2.4. Data analysis

The effect of each antagonist was determined by comparing the ED_{50} for the hypotensive effect of adenosine in the presence and absence of the antagonist, using a computer-generated multiple comparison test (MULTCOMP). The ED_{50} value was taken as the dose required to produce 50% of the maximal response of adenosine. A *P*-value of less than 0.05 was considered to indicate statistical significance. ED_{50} values with their associated confidence limits were calculated using a computer program (Tallarida).

3. Results

3.1. The cardiovascular actions of adenosine in the anaesthetized and pithed rat preparation

Bolus doses of adenosine in the range 10–3000 μg/kg produced a dose-dependent decrease in diastolic blood pressure. Adenosine induced decreases in blood pressure were attenuated by each of the adenosine receptor antagonists used. Table 1 shows the ED_{50} values for adenosine in the presence of 50 μg/kg/min infusions of DPCPX, 8PT, 8SPT and DMPX.

The maximum increase in ED_{50} (11.3-fold) was caused by a 50 μg/kg/min infusion of DPCPX. The effect of DPCPX on responses to adenosine was significantly greater than that of 8PT ($n = 6$, $P < 0.05$). The combined uptake inhibitors caused a significant decrease in ED_{50} for the adenosine dose-response curve (2.4-fold, $n = 5$, $P < 0.05$, see Table 1). Resting blood pressure was not altered by infusion of the uptake inhibitors, adenosine receptor antagonist infusion, naloxone, mepyramine or atropine. Guanethidine (16 μg/kg/min), naloxone (10 mg/kg), mepyramine (10 μg/kg/min) and atropine (1 mg/kg) all had no significant effect on the hypotensive response to adenosine ($n = 6$, $P > 0.05$, see Table 1). Resting blood pressure values for guanethidine-infused animals were 89.1 ± 9.7 mm Hg, which was not significantly different to that of control animals ($n = 6$, $P > 0.05$).

Responses to adenosine did not significantly alter over the course of the experiment. The antagonist vehicle (10% DMSO in saline) had no significant effect on the adenosine dose-response curve.

DMPX (50 μg/kg/min) and 8SPT (50 μg/kg/min) caused significant shifts ($n = 5$, $P < 0.05$) to the right of the adenosine dose-response curve, whilst DPCPX and 8PT had no significant effect on response to adenosine in pithed rats ($P > 0.05$). The adenosine uptake inhibitors produced a significant leftward shift of the dose-response curve ($n = 6$, $P < 0.05$) in the pithed rat.

3.2. The cardiovascular actions of morphine in the anaesthetized and pithed rat preparation

Bolus doses of morphine in the range 10–1000 μg/kg produced decreases in diastolic blood pressure, with a 1000 μg/kg dose resulting in an 83.1 ± 7.2% ($n = 10$) decrease in diastolic blood pressure. Tolerance to the cardiovascular effects of morphine developed rapidly. In preliminary experiments it was found that limiting the total number of doses of morphine to four (two before and two after infusion of antagonist or vehicle) and allowing at least 20 min between doses gave the most consistent results with no time-related decrease in the response to morphine being seen. The hypotensive action of morphine (100 μg/kg) was attenuated by 8PT and DPCPX (50 μg/kg/min) to give 68.9 ± 11.2 and 81.6 ± 8.4% reductions ($n = 6$, $P < 0.05$) of responses observed in control animals. DPCPX was significantly more effective than 8PT at attenuating the decrease in blood pressure produced by 30 and 100 μg/kg morphine ($n = 6$, $P < 0.05$). 8SPT and DMPX (50 μg/kg/min) had no effect on morphine

Table 1
The effect of different adenosine receptor antagonists (50 μg/kg/min), an adenosine uptake inhibitor combination, guanethidine (16 μg/kg/min), atropine (1 mg/kg), naloxone (10 mg/kg) and mepyramine (10 μg/kg/min) on adenosine induced decreases in diastolic blood pressure in anaesthetized and pithed rats

Antagonist	Anaesthetized	Pithed
Control	91.96 ± 4.3	927.9 ± 99.4
DPCPX	984.8 ± 99.1[a]	876.5 ± 228.8
8PT	922.6 ± 65.5[a]	921.0 ± 186.2
8SPT	383.8 ± 59.4[a]	1363.0 ± 204.9[b]
DMPX	329.7 ± 55.8[a]	1286.3 ± 163.9[b]
Uptake inhibitors	38.3 ± 7.5[a]	27.3 ± 2.73[b]
Guanethidine	110.7 ± 19.1	906.5 ± 101.2
Atropine	89.9 ± 11.2	886.4 ± 86.8
Naloxone	99.1 ± 4.7	902.5 ± 101.9
Mepyramine	98.2 ± 10.4	961.6 ± 138.3

All values are ED_{50} (μg/kg) ± C.L. (95% confidence limit) for the effect of adenosine on blood pressure in the presence of 50 μg/kg/min of the four adenosine receptor antagonists ($n = 5$–10 for each value). [a]$P < 0.05$ vs. control; [b]$P < 0.05$ vs. control. The uptake inhibitor combination consisted of 0.5 μg/kg/min dipyridamole, 0.82 μg/kg/min NBTI and 2.5 μg/kg/min lidoflazine.

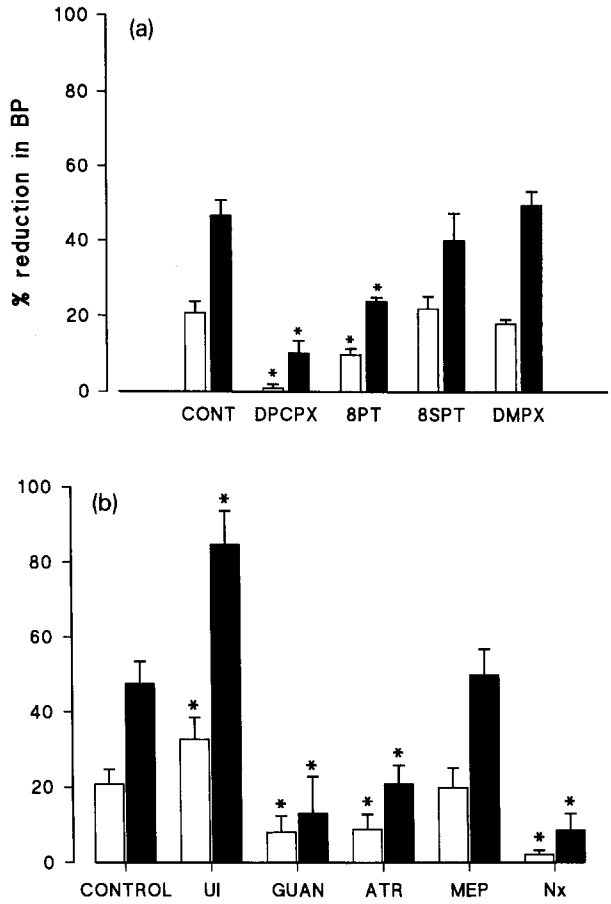

Fig. 1. (a) Effect of the adenosine receptor antagonists (50 μg/kg/min) on depressor responses to morphine 30 μg/kg (□) and 100 μg/kg (■) in anaesthetized rats (mean ± S.E.M., $n = 5$–10). * Significant difference from the control values; ANOVA, $P < 0.05$. (b) Effect of the uptake inhibitor combination (UI, 0.5 μg/kg/min dipyridamole, 0.82 μg/kg/min NBTI and 2.5 μg/kg/min lidoflazine), guanethidine (GUAN, 16 μg/kg/min), atropine (ATR, 1 mg/kg), mepyramine (MEP, 10 μg/kg/min) and naloxone (Nx, 10 mg/kg) on responses to morphine 30 μg/kg (□) and 100 μg/kg (■) in anaesthetized rats (mean ± S.E.M., $n = 5$–10). * Significant difference from the control values; ANOVA, $P < 0.05$.

induced decreases in blood pressure. These data are summarised in Fig. 1a. An infusion of the uptake inhibitor combination induced a significant increase in the hypotensive action of morphine (100 μg/kg), to produce a response 1.81-fold greater than that observed in control animals ($n = 5$; $P < 0.05$; Fig. 1b). Atropine (1 mg/kg) attenuated the hypotensive response to 100 μg/kg morphine (Fig. 1b). Mepyramine (10 μg/kg/min) infusion did not affect the response to morphine (30 and 100 μg/kg, $n = 10$), whilst naloxone (10 mg/kg) significantly attenuated the hypotensive response to morphine (88.4 ± 11.2% reduction of initial, $n = 10$; $P < 0.05$, Fig. 1b). Guanethidine infusion (16 μg/kg/min) resulted in a decrease in response to morphine at 30 and 100 μg/kg (Fig. 1b). A suppression of the hypertensive reflex response to a carotid artery occlusion during guanethidine infusion was also observed.

Morphine administration had no effect on blood pressure in pithed rats infused with phenylephrine (or those tested without phenylephrine infusion) at doses up to 10 mg/kg.

4. Discussion

The experiments which are described here illustrate that in the anaesthetized rat, intravenous doses of both morphine and adenosine cause falls in blood pressure. In most instances, the responses to morphine were more prolonged than those to adenosine. This can be explained by the fact that adenosine is rapidly inactivated by both uptake into tissues and enzymatic degradation (see review by Williams (1989) for an account of adenosine inactivation).

The ability of the adenosine receptor antagonists to inhibit depressor responses to both morphine and adenosine suggests that the effects of both drugs are mediated by activation of adenosine receptors. Both adenosine A_1 and A_2 receptors were involved in mediating the hypotensive response to adenosine, as antagonists of both receptor types caused a reduction in the effects of adenosine. DPCPX is a highly selective adenosine A_1 receptor antagonist, with the other antagonists 8PT, 8SPT, and DMPX being progressively more selective for A_2 receptors. DPCPX and 8PT were the only adenosine receptor antagonists able to inhibit hypotensive responses to morphine, with DPCPX being significantly more effective. In this study, 8PT and DPCPX were used because, in intact tissues, 8PT is non-selective for adenosine A_1 and A_2 receptors, whilst DPCPX has a 30- to 50-fold greater affinity for A_1, but is equi-effective with 8PT at adenosine A_2 receptors (Collis et al., 1989). As DPCPX was significantly more effective at attenuating the hypotensive response to morphine than 8PT, we suggest that adenosine A_1 receptors may be involved in mediating the hypotensive response to morphine.

Furthermore, the location of these receptors appears to be predominantly central since DPCPX blocked the hypotensive response to morphine whilst 8SPT (at a dose which attenuated the hypotensive response to adenosine) was ineffective. Evonuik et al. (1986) showed that following intravenous administration of a 50 mg/kg bolus dose of 8SPT, the drug could not be detected in the central nervous system, suggesting that the drug is confined to the peripheral circulation. Therefore 8SPT may be considered as a nonselective, peripherally acting adenosine receptor antagonist. In the experiments described here the lack of effect of 8SPT on responses to morphine provides evidence that adenosine A_1 receptors located at the sympathetic neuroeffector junction do not make a major contribution to the hypotensive effects of morphine. It has also been reported that the quaternary analogue of morphine, morphine-methyl-iodide, does not affect blood pressure in anaesthetized rats (Calignano et al., 1992) which supports the argument that the action of morphine is mediated centrally.

Three adenosine uptake inhibitors (lidoflazine, dipyridamole and NBTI) were used in combination, as it has been reported that this combination produces the most effective inhibition of adenosine uptake (Ballarin et al., 1991). The uptake inhibitor combination potentiated the hypotensive effect of adenosine in anaesthetized rats, confirming that an effective inhibition of adenosine uptake was achieved with these drugs. The adenosine uptake inhibitors also enhanced the hypotensive response to morphine, suggesting that adenosine release is involved in mediating this effect of morphine. The potentiation of the effect of adenosine on blood pressure by adenosine uptake inhibitors was greater than the potentiation of the effect of morphine. This is possibly due to greater concentration of the uptake inhibitors at the site of action. As the hypotensive effect of adenosine is largely peripherally mediated, and the effect of morphine appeared to be centrally mediated, it is likely that a greater concentration of adenosine is present peripherally than that reaching the site of action of morphine in the brain.

Guanethidine infusion reduced the effect of morphine on blood pressure, and therefore it is likely that the hypotensive effect of morphine is due at least in part to a decrease in sympathetic tone via release of adenosine. Since guanethidine was shown to cause suppression of the sympathetically mediated carotid artery occlusion reflex it is clear that sympathetic neurotransmission was reduced. Responses to adenosine were not significantly reduced by guanethidine infusion, which was not unexpected considering the direct vasodilator effect of adenosine. Atropine also effectively attenuated the hypotensive response to mor-

phine, implicating a parasympathetic component to the hypotensive response to morphine.

In conclusion, the results presented here suggest that the hypotensive effects of morphine are mediated by adenosine released in the central nervous system and possibly acting at adenosine A_1 receptors to cause a decrease in sympathetic tone. Further experiments to confirm the adenosine receptor subtype involved are currently in progress.

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