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Changes in adenosine receptors mediating hypotension in morphine-dependent rats

Paul J. White *, Roselyn B. Rose'Meyer, Wendy Hope

Department of Pharmaceutical Biology and Pharmacology, Victorian College of Pharmacy, Monash University, Parkville, Victoria 3052, Australia

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Abstract

The hypotensive actions of morphine have been shown to be mediated by adenosine. Since tolerance has been reported to the hypotensive effects of morphine, this study was designed to determine whether morphine dependence altered adenosine receptor-mediated decreases in blood pressure in the Hooded Wistar rat. Following the induction of morphine dependence, the effects of adenosine receptor agonists and antagonists were studied in intact and pithed rat preparations. The hypotensive effects of adenosine were significantly less in morphine-dependent rats when compared to opiate naive rats. The adenosine A_1 receptor agonist cyclohexyladenosine induced decreases in diastolic blood pressure which were significantly reduced in morphine-dependent rats when compared to opiate naive rats. However, the adenosine A_{2A} receptor agonist 2-*p*-(carboxyethyl)-phenylamino-5'-*N*-ethylcarboxamidoadenosine (CGS 21680) had a greater effect on blood pressure in morphine-dependent rats compared to opiate naive rats. The effects of adenosine receptor antagonists 8-cyclopentyl-1,3-dipropylxanthine, 8-phenyltheophylline, 8-(*p*-sulfophenyl)theophylline and 3,7-dimethyl-1-propargylxanthine infused at 50 μg/kg per min on the hypotensive actions of adenosine were studied in opiate naive and morphine-dependent rats. In intact rats the induction of morphine dependence reduced the potency of these antagonists at inhibiting adenosine-induced decreases in blood pressure. The same series of experiments was conducted in the pithed rat preparation. In this case the hypotensive actions of both cyclohexyladenosine and CGS 21680 were greater in morphine-dependent rats than opiate naive rats. In pithed rats, morphine dependence did not change the potencies of adenosine receptor antagonists on the hypotensive actions of adenosine. These results suggest that adenosine A_1 receptors are downregulated in morphine-dependent rats, and that adenosine A_2 receptors are upregulated in morphine-dependent rats.

Keywords: Adenosine; Morphine dependence; Blood pressure

1. Introduction

Morphine produces both hypotension and bradycardia when administered to anaesthetized rats (Fennessy and Rattray, 1971; Calignano et al., 1992). As with other opioid effects, tolerance to the cardiovascular actions of morphine develops rapidly with repeated exposure (White et al., 1993). The mechanisms underlying tolerance and dependence are poorly understood, and research is usually directed at the analgesic rather than cardiovascular effects of morphine. The effects of chronic exposure to morphine on cardiovascular parameters have not been widely studied.

The cardiovascular effects of morphine have been postulated to be mediated by the release of adenosine. Methylxanthines, adenosine receptor antagonists, block a wide range of opiate actions, including analgesia (Sawynok et al., 1991) and morphine has been shown to release endogenous adenosine from the spinal cord (Sweeney et al., 1987). Calignano et al. (1992) found that methylxanthines could block the hypotensive actions of morphine, and previous work in this laboratory suggested that adenosine release followed by inhibition of sympathetic tone was involved in mediating the hypotensive actions of morphine (White et al., 1993).

Morphine-induced decreases in blood pressure have been shown to be produced by a central action at the nucleus of the tractus solitarii as microinjection of morphine into this brain region results in hypotension and bradycardia (Tseng et al., 1988). Adenosine pro-

* Corresponding author. School of Pharmacology, Victorian College of Pharmacy, 381 Royal Pde, Parkville 3052, Australia. Tel.: (03) 903 9566; fax: (03) 903 9638.

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duces hypotension and bradycardia by both central and peripheral mechanisms (Berne, 1980). Adenosine has similar effects to morphine when microinjected into the nucleus tractus solitarii (Mosqueda-Garcia et al., 1991), but also has direct effects on the heart and blood vessels to produce decreases in heart rate and blood pressure. The nucleus tractus solitarii has been shown to be an important site for the co-ordination of cardiorespiratory response patterns (Jordan and Spyer, 1986) and hence any changes to the adenosine receptor mechanisms in this region may alter cardiovascular responses to changes in blood pressure.

Given that tolerance occurs to the hypotensive response to morphine, and that adenosine mediates the effects of morphine, it is possible that changes to adenosine receptor mechanisms may occur in response to chronic exposure to morphine. Therefore, the purpose of this study was to examine the effects of chronic exposure to morphine on hypotensive responses to adenosine receptor agonists and antagonists. Considering the different sites of action of adenosine, an attempt was made to differentiate between central and peripheral changes in response to chronic opiate exposure.

2. Materials and methods

2.1. Animals

Female Hooded Wistar rats weighing 205–320 g were housed in North Kent Plastics Cages with sawdust bedding. Animals were provided with tap water and food in the form of Clark King ARM cubes ad libitum. The room in which the animals were housed was maintained at 18–20°C on a 12 h light : 12 h dark cycle. Animals were divided into four experimental groups: intact opiate naive, intact morphine-dependent, pithed opiate naive and pithed morphine-dependent.

2.2. Anaesthetized (intact) rat preparation

Animals 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. The jugular and femoral vein cannulae were rinsed with physiological saline, while the carotid cannula was flushed with an heparinized saline solution (100 UI heparin/ml saline). Blood pressure from the carotid was recorded using a Gould State Physiological pressure transducer connected to a Grass 79D polygraph recorder. Following dissection the rat was left for 30 min to allow equilibration of the preparation.

2.3. Pithed rats

Rats were prepared as above prior to pithing. A metal rod was inserted through the orbit of the eye, and pushed into the brain. Lateral movements of the rod in the brain were performed to ensure that both sides of the brain were destroyed. The rod was then pushed into and along the spinal cord. A Parvalux respirator was connected to the tracheal cannula (63 breaths/min, 4.0 ml/breath) once spontaneous breathing stopped. The animal was then atropinised (0.1 mg/kg) to prevent accumulation of excess bronchial secretion. Phenylephrine (0.2 mg/kg per min) was infused via the femoral vein using a Palmer injection apparatus to elevate the rat blood pressure to a level which allowed measurement of depressor responses (121 ± 22.5 mm Hg), since pithing resulted in a resting blood pressure of around 45 mm Hg. Vasodilator doses were given at 3 min intervals in the pithed rats as the blood pressure could not be maintained for more than 20 min. Results were expressed as a percentage decrease of the phenylephrine-elevated blood pressure.

2.4. Morphine-dependent rats

Animals were made morphine-dependent using the method described by Dionyssopoulos et al. (1992). Morphine base was formulated into an emulsion (saline : liquid paraffin : mannide monooleate, 8 : 6 : 1). Animals were injected (s.c.) in the scruff of the neck with a total of 250 mg/kg morphine in a volume of 0.5 ml/100 g. Half the dose was administered at 0 h and the remainder at 24 h. At 48 h the animal was used as an intact or pithed rat preparation as previously described.

2.5. Protocol

Responses to morphine and adenosine were determined in each group of animals. A dose-response curve to adenosine was constructed in each animal, and 30 and 100 mg/kg doses of morphine were tested in each animal. The effects of adenosine receptor agonists cyclohexyladenosine and CGS21680 were examined in rats from each group. The effects of a series of adenosine receptor antagonists on hypotensive responses to adenosine were determined. The antagonists at 50 μg/kg per min were infused via the femoral vein for 15 min prior to, and continued during, the second dose-response curve. Only one antagonist at one dose was used in each animal. Experiments were carried out using a vehicle infusion. Changes in diastolic blood

pressure were measured to estimate changes in peripheral resistance. Results were expressed as a percentage decrease of the resting diastolic blood pressure.

2.6. Drugs

Drugs used included phenylephrine hydrochloride (Sigma), adenosine, 8-cyclopentyl-1,3-dipropylxanthine, 8-phenyltheophylline, 8-(*p*-sulfophenyl)theophylline, 3,7-dimethyl-1-propargylxanthine, N^6-cyclohexyladenosine, 2-*p*-(carboxyethyl)phenylamino-5'-*N*-ethylcarboxamidoadenosine (CGS 21680) (Research Biochemicals), morphine hydrochloride (Macfarlane Smith), and pentobarbitone sodium (Boehringer Ingelheim). All drugs were dissolved in 0.75% NaOH and 1% dimethylsulfoxide (DMSO) and diluted to required concentration in saline.

2.7. Statistical analysis

The effect of each antagonist was determined by comparing the ED_{50} for the hypotensive effect of adenosine in the presence of the antagonist with that of adenosine alone, using a computer-generated multiple comparison test (MULTCOMP). The ED_{50} was taken as the dose of adenosine required to produce 50% of the maximal response of adenosine. MULTCOMP was also used to compare the effect of the adenosine agonists on blood pressure in control and morphine-dependent rats. A P value of less than 0.05 was considered to indicate statistical significance. ED_{50} values and their confidence intervals were calculated using a computer program, Tallarida.

3. Results

3.1. Responses to morphine and adenosine in intact opiate naive and intact morphine-dependent rats

In intact anaesthetized rats bolus doses of morphine in the range 10–1000 μg/kg produced decreases in blood pressure, with a 100 μg/kg dose resulting in an 83.1 ± 7.2% ($n = 10$) decrease in diastolic blood pressure. Morphine had no effect on blood pressure in doses up to 10 mg/kg in the pithed rats, morphine-dependent rats and pithed morphine-dependent rats. Representative traces showing the effect of morphine and adenosine on blood pressure in each of the experimental groups are shown in Fig. 1.

Adenosine (30–10000 μg/kg) produced dose-dependent decreases in diastolic blood pressure in the four animal groups tested. All three groups of treated rats (intact morphine-dependent, pithed, and pithed morphine-dependent) were less sensitive to adenosine

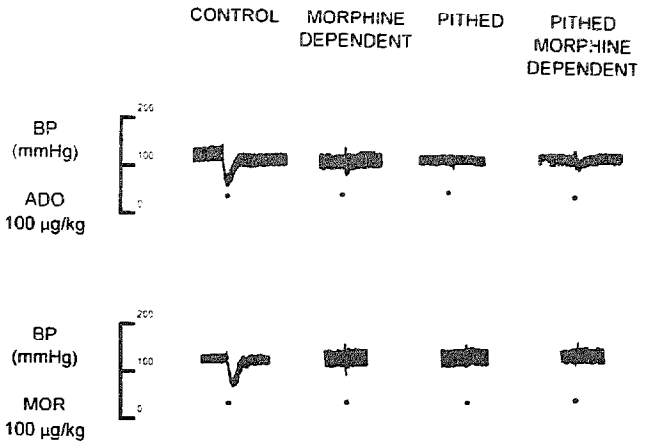

Fig. 1. Experimental traces showing vascular responses to morphine (MOR, 100 μg/kg) and adenosine (ADO, 100 μg/kg) in intact opiate naive (control), intact morphine-dependent (morphine-dependent), pithed opiate naive (pithed) and pithed morphine-dependent rats.

than intact opiate naive rats ($n = 10$, $P < 0.05$). Intact opiate naive rats were more sensitive to adenosine than intact morphine-dependent rats ($n = 10$, $P < 0.05$). Pithed, morphine-dependent rats were significantly more sensitive to adenosine than pithed, opiate-naive rats ($n = 10$, $P < 0.05$). These data are illustrated in Fig. 2.

3.2. The effects of morphine dependence on hypotensive responses to adenosine analogues in intact and pithed rats

The adenosine A_1 receptor-selective agonist cyclohexyladenosine induced dose-dependent decreases in diastolic blood pressure in opiate naive and morphine-dependent rats. Responses to cyclohexyladenosine were significantly attenuated in intact morphine-dependent rats when compared to intact opiate naive rats ($n = 5$, $P < 0.05$). A 10 μg/kg dose of cyclohexyladenosine produced a 58.4 ± 18.2% decrease in diastolic blood pressure in opiate naive rats, whilst a 12.2 ± 5.7% decrease was observed in morphine-dependent rats. Fig. 3 shows the effect of cyclohexyladenosine (3 and 10 μg/kg) on blood pressure in opiate naive and morphine-dependent rats.

CGS 21680, an adenosine A_2 selective agonist, also induced dose-dependent decreases in diastolic blood pressure in opiate naive and morphine-dependent rats (see Fig. 4). Intact morphine-dependent rats were more sensitive to the vasodilator effects of CGS 21680 than intact opiate naive rats ($n = 5$, $P < 0.05$).

Responses to cyclohexyladenosine (3 and 10 μg/kg) were greater in pithed morphine-dependent rats when compared to pithed opiate naive rats ($n = 6$, $P < 0.05$, see Fig. 3). In the pithed rat preparation CGS 21680-induced decreases in blood pressure were also signifi-

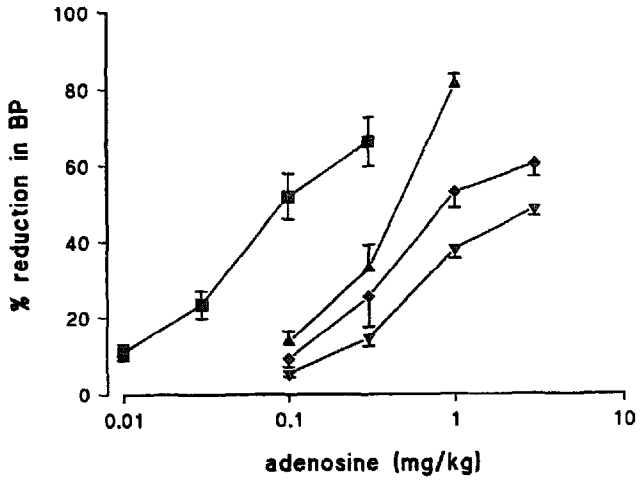

Fig. 2. Dose-response curves to adenosine in the different treatment groups. Intact: ■; intact morphine-dependent: ▲; pithed: ▼; pithed morphine-dependent: ◆.

cantly greater in morphine-dependent rats compared to opiate naive ($n = 6$, $P < 0.05$, see Fig. 4).

3.3. The effect of adenosine receptor antagonists on responses to adenosine in intact and pithed rats

Table 1 shows the ED_{50} values for the hypotensive response to adenosine in the presence of each of the adenosine receptor antagonists for intact opiate naive and morphine-dependent rats. The antagonists 8-phenyltheophylline, 8-(*p*-sulfophenyl)-theophylline and 3,7-dimethyl-1-propargylxanthine (50 μg/kg per min) attenuated the response to adenosine in both opiate

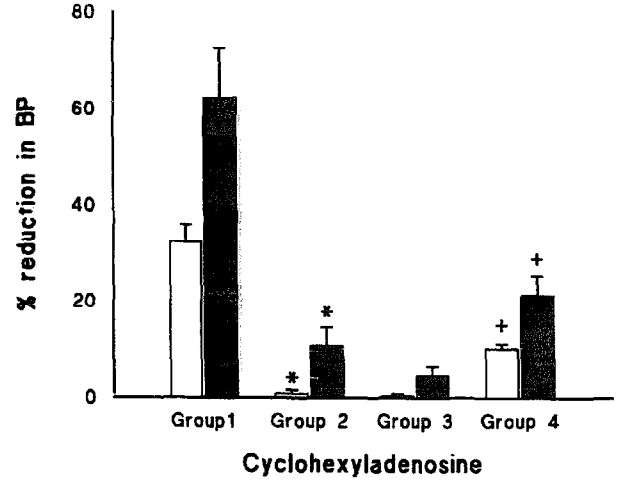

Fig. 3. Effect of cyclohexyladenosine 3.0 μg/kg (open columns) and 10.0 μg/kg (filled columns) on blood pressure in intact opiate naive (group 1), intact morphine-dependent (group 2), pithed opiate naive (group 3) and pithed opiate-dependent (group 4) rats (means± S.E.M., $n = 10$). * Indicates a significant difference between intact opiate naive and intact morphine-dependent rats. + Indicates a significant difference between pithed opiate naive and pithed morphine-dependent rats; ANOVA, $P < 0.05$.

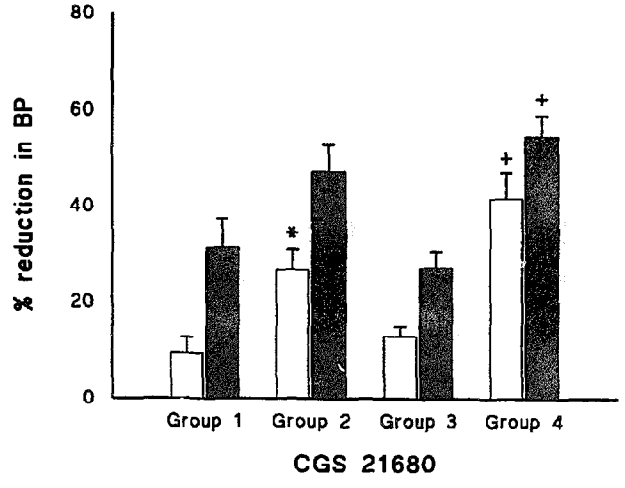

Fig. 4. Effect of CGS 21680 100.0 μg/kg (open columns) and 300.0 μg/kg (filled columns) on blood pressure in intact opiate naive (group 1), intact morphine-dependent (group 2), pithed opiate naive (group 3) and pithed opiate-dependent (group 4) rats (means± S.E.M., $n = 10$). * Indicates a significant difference between intact opiate naive and intact morphine-dependent rats. + Indicates a significant difference between pithed opiate naive and pithed morphine-dependent rats; ANOVA, $P < 0.05$.

naive and morphine-dependent rats ($P < 0.05$). The degree of rightward shift in the adenosine dose-response curves produced by the antagonists was reduced in morphine-dependent animals. In opiate naive rats 8-phenyltheophylline produced a 10.1-fold shift compared to a 2.6-fold shift in morphine-dependent animals. Similar results were observed for 8-(*p*-sulfophenyl)-theophylline where in opiate naive animals a 4.2-fold shift was produced which was significantly greater than the 1.7-fold shift in morphine-dependent animals. For the antagonist 3,7-dimethyl-1-propargylxanthine no significant differences between shifts in adenosine dose-response curves in opiate naive and morphine-dependent rats were observed ($n = 5$,

Table 1
The effect of adenosine receptor antagonists: 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), 8-phenyltheophylline (8PT), 8-(*p*-sulfophenyl)-theophylline, 3,7-dimethyl-1-propargylxanthine (DMPX) (50 μg/kg per min) on the hypotensive effects of adenosine in intact rats

	Control		Morphine-dependent	
	ED_{50} (μg/kg)	Shift	ED_{50} (μg/kg)	Shift
No antagonist	91.9±4.4	1.0	696.0±139.5	1.0
8PT	822.6±165.5 [a]	9.0	1824.5±397.9 [b]	2.6
DPCPX	984.8±199.1 [a]	10.7	600.5±136.5	0.87
8SPT	383.8±139.4 [a]	4.2	1154.4±230.3 [b]	1.7
DMPX	245.8±39.9 [a]	2.7	1187.9±229.7 [b]	1.7

Values are ED_{50} (μg/kg)±C.I. for $n = 5$–7 animals per group. [a] $P < 0.05$ vs. no antagonist-control, [b] $P < 0.05$ vs. no antagonist morphine-dependent. Shift represents the ED_{50} value for that group as a fraction of the no antagonist ED_{50}.

Table 2
The effect of adenosine receptor antagonists: 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), 8-phenyltheophylline (8PT), 8-(*p*-sulfophenyl)-theophylline, 3,7-dimethyl-1-propargylxanthine (DMPX) (50 μg/kg per min) on the hypotensive effects of adenosine in pithed rats

	Control		Morphine-dependent	
	ED_{50} (μg/kg)	Shift	ED_{50} (μg/kg)	Shift
No antagonist	927.9 ± 99.4	1.0	706.7 ± 83.4	1.0
8PT	1824.5 ± 186.2 [a]	2.0	1726.7 ± 262.8 [b]	2.4
DPCPX	876.5 ± 128.8 [a]	0.94	610.4 ± 162.5	0.86
8SPT	1363.0 ± 204.9 [a]	1.4	1154.4 ± 230.3 [b]	1.7
DMPX	1986.3 ± 163.8 [a]	2.1	1275.1 ± 125.5 [b]	1.8

Values are ED_{50} (μg/kg) ± C.I. for $n = 5$–7 animals per group. [a] $P < 0.05$ vs. no antagonist-control, [b] $P < 0.05$ vs. no antagonist morphine-dependent. Shift represents the ED_{50} value for that group as a fraction of the no antagonist ED_{50}.

$P > 0.05$). 8-Cyclopentyl-1,3-dipropylxanthine, whilst producing a 10.7-fold shift in adenosine ED_{50} in opiate naive rats, was ineffective in morphine-dependent rats.

Table 2 shows the effect of the adenosine receptor antagonists on responses to adenosine in pithed opiate naive and morphine-dependent rats. 8-Cyclopentyl-1,3 dipropylxanthine (50 μg/kg per min) caused no significant inhibition of adenosine-induced decreases in diastolic blood pressure ($n = 5$, $P > 0.05$). 3,7-Dimethyl-1-propargylxanthine and 8-(*p*-sulfophenyl)-theophylline produced significant inhibition of vascular responses to adenosine in both groups of rats ($n = 5$, $P < 0.05$). The degree of shifts in the adenosine dose-response curves for 3,7-dimethyl-1-propargylxanthine and 8-(*p*-sulfophenyl)-theophylline were not significantly different in opiate naive and morphine-dependent animals.

4. Discussion

Morphine induced dose-dependent decreases in diastolic blood pressure in the anaesthetized rat preparation, but had no effect on blood pressure in pithed or morphine-dependent animals. These results indicate that morphine requires an intact central nervous system (CNS) to induce a decrease in blood pressure and that tolerance occurs to the hypotensive action of morphine. Adenosine, however, produced decreases in blood pressure in all four preparations used. In intact rats, exogenous adenosine may act on receptors located on vascular smooth muscle (A_2, Berne, 1980), prejunctional receptors on sympathetic nerves, or directly on the heart (A_1, Evans et al., 1982). In pithed rats, however, only the adenosine receptors located on the vascular smooth muscle and possibly the heart could mediate effects on blood pressure. It is unlikely that adenosine receptors in the CNS play a role in decreases in blood pressure caused by exogenous adenosine, as adenosine has been shown to be effectively removed after entering the CNS by rapid enzymatic breakdown (Pardridge et al., 1994). The adenosine analogues used are more stable, however, and may exert central as well as peripheral effects.

Our investigation into the effects of adenosine on the blood pressure of morphine-dependent rats showed that significant changes in responses to adenosine occur depending on whether the morphine-dependent animals are pithed or intact. In intact, opiate naive animals, responses to adenosine were greater than in intact morphine-dependent rats, whilst in pithed animals, responses to adenosine were greater in the morphine-dependent group than the opiate naive group. These results suggest that opposing changes to adenosine receptors may occur with morphine dependence.

To study effects on adenosine receptors further, selective agonists were implemented. The adenosine A_1 receptor agonist cyclohexyladenosine and A_{2A} adenosine receptor agonist CGS 21680 were used in the various rat preparations. Cyclohexyladenosine has been shown to have approximately 400-fold selectivity for adenosine A_1 receptors over adenosine A_2 receptors in rat brain membranes (Bruns et al., 1986). CGS 21680 is a highly selective adenosine A_2 receptor agonist, with 170–1500-fold selectivity for A_2 receptors over A_1 (Hutchison et al., 1990), and is the most potent adenosine receptor agonist at A_{2A} receptors (Collis and Hourani, 1993). In intact rats, decreases in diastolic blood pressure observed in response to cyclohexyladenosine and CGS 21680 can be due to activation of central and peripheral receptors. In the intact rat preparation responses to cyclohexyladenosine were reduced in morphine-dependent rats, while in pithed rat preparations the hypotensive actions of cyclohexyladenosine were increased with morphine dependence. In both intact and pithed rat preparations CGS 21680 was found to be more potent in morphine-dependent animals. These results suggest that adenosine A_1 receptors may be downregulated and adenosine A_2 receptors upregulated following morphine dependence.

In pithed rats the central component of the hypotensive response has been removed, leaving only peripheral mechanisms. The fact that pithed morphine-dependent rats were more sensitive to the hypotensive actions of adenosine, CGS 21680 and cyclohexyladenosine when compared to control animals suggests that significant increases in adenosine receptor affinity or number has occurred, most likely at the vascular smooth muscle adenosine receptors.

Changes in adenosine receptors in morphine-dependent rats were also demonstrated using adenosine receptor antagonists. 8-Cyclopentyl-1,3-dipropylxanthine, an adenosine A_1 receptor selective antagonist (Lohse et al., 1987) attenuated hypotensive responses to adenosine in intact opiate naive rats but not in pithed

or morphine-dependent rats. These results suggest a lack of effective adenosine A_1 receptors in the hypotensive actions of adenosine in pithed and morphine-dependent rats.

These findings can be supported by binding studies carried out by Ahlijanian and Takemori (1986), who reported that adenosine A_2 receptors were upregulated (increased in number) after chronic exposure to morphine, whilst Tao and Lui (1992) found that chronic morphine treatment causes downregulation of spinal adenosine A_1 receptors in rats. Changes observed in morphine-dependent animals may involve the adenylyl cyclase second messenger system, as opiate receptors are frequently negatively coupled to this enzyme (Benalal and Bachrach, 1985). During the development of morphine tolerance and dependence, a persistent inhibition of adenylate cyclase occurs. In response to this, opiate sensitive neuroblastoma cells have been shown to adapt by increasing the activity of adenylate cyclase, and it has been suggested that the ability of adenylate cyclase to hypertrophy is the underlying mechanism of tolerance and dependence upon opiates (Barchfield et al., 1982). Opposing effects upon adenosine A_1 and A_2 receptors after chronic exposure to morphine may be explained in terms of their effects on the adenylate cyclase second messenger system. Adenosine A_1 receptors are known to be negatively coupled to adenylate cyclase, whilst A_2 receptors are positively coupled to the same enzyme (Van Caulker et al., 1979). Hence the increase in number of A_2 receptors that occurs during the development of tolerance to morphine may be a compensatory response to the persistent inhibition of adenylate cyclase that accompanies chronic morphine exposure.

The overall result of these changes to adenosine receptor mechanisms in intact morphine-dependent rats is a decrease in sensitivity to adenosine, and therefore it is possible that the downregulation of adenosine A_1 receptors has a larger influence than the upregulation of adenosine A_2 receptors. Any changes to the sensitivity of adenosine may have important consequences with respect to control of the cardiovascular system, as adenosine has been reported to have a role as an autoregulator in this area (Berne et al., 1980).

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