Arterial catecholamine levels in morphine-treated rats subjected to sympathetic nerve stimulation

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- 1 The effect of acute or chronic morphine treatment on the changes in arterial noradrenaline and adrenaline levels in response to sympathetic nerve stimulation was studied in rats.
- 2 Rats which had been chronically treated with morphine in their drinking fluid for 21 days were shown to be morphine-tolerant, as revealed by the tail-immersion test for analgesia.
- 3 It was found that animals given either acute or chronic morphine treatment had similar basal concentrations of arterial catecholamines to their controls.
- 4 Sympathetic nerve stimulation produced significant increases in arterial noradrenaline and adrenaline levels in both the control and morphine-treated animals. However, the degree of arterial noradrenaline elevation was significantly less in morphine-tolerant animals.
- 5 This phenomenon was not observed in acutely morphine-treated rats or at 2 weeks following opiate withdrawal in animals which had been treated previously with morphine for 3 weeks.
- 6 The findings suggest that chronic morphine treatment in rats not only leads to opiate tolerance but also reduces catecholamine release in response to sympathetic nerve stimulation.

Introduction

Although it has been postulated that morphine and many other opioids may influence the release of neurotransmitters in opiate-sensitive neuronal tissues, their mechanism of action is not clear. In most studies, their effects on the central or peripheral nervous systems were deduced from isolated tissue preparations or cell cultures (Snyder, 1978; Terenius, 1978; Beaumont & Hughes, 1979). There are relatively few studies on peripheral sympathetic function in whole animals which have been acutely or chronically treated with morphine (Jurna & Rummel, 1984), or on the profile of catecholamine release following peripheral sympathetic nerve stimulation in morphine-treated animals (Wada *et al.*, 1938; Radosevich *et al.*, 1984).

Prolonged morphine administration is known to produce physical dependence and tolerance. It has been reported that, depending on the species and opioid-exposure time, experimental animals also exhibit other physiological alterations after chronic opiate treatment which are different from those observed after acute treatment (Schulz & Goldstein, 1973; Kromer & Steigemann, 1982; Rae & De Moraes, 1983; Dai *et al.*, 1985; Martin & Takemori,

1986). Recently, it has been observed that chronically morphine-treated rats exhibit a significantly lower incidence and delayed onset of early ventricular arrhythmias, and a marked decline in ventricular noradrenaline concentration, during acute myocardial ischaemia (Chan *et al.*, 1987; Ko *et al.*, 1988). A previous study has shown that the cardiovascular responses to sympathetic nerve stimulation are significantly reduced following prolonged morphine administration (Leung *et al.*, 1986b). Thus, it is possible that chronic opiate treatment may influence the alterations in arterial catecholamine levels elicited by sympathetic nerve stimulation.

The present study examines the effects of sympathetic nerve stimulation on arterial noradrenaline (NA) and adrenaline (Ad) levels in acutely or chronically morphine-treated rats, and in a group of animals from which morphine had been withdrawn for 2 weeks after receiving morphine for 3 weeks.

Methods

Animals

Female Sprague–Dawley rats, weighing 140–160 g, were fed a standard rat pellet diet (Ralston Purina

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Co., U.S.A.) and given tap water to drink *ad libitum*. They were housed in a temperature ($23 \pm 1^\circ\text{C}$) and humidity ($65 \pm 5\%$)-controlled room, and were exposed to a 12 h light/12 h dark cycle.

Morphine treatment

In the studies on the effects of acute morphine treatment, animals were injected i.p. with an analgesic dose of morphine sulphate (Macfarlan Smith, Middlesex) 16 mg kg^{-1} , expressed as the salt, 15 min before sympathetic nerve stimulation. Control rats were given a solution of 0.9% w/v NaCl (saline) 1 ml kg^{-1} by the same route and at the same time.

In the case of chronic morphine treatment, the opiate was administered in the drinking fluid for 21 days. A solution of 5% w/v sucrose in ordinary tap water was used as drinking fluid in order to mask the bitter taste of morphine (Dai *et al.*, 1984). Morphine sulphate, weight expressed as the salt, was given in increasing concentrations (48 h apart) of 0.1, 0.2, 0.3 and finally 0.4 mg ml^{-1} . The rats continued to receive the final concentration until the end of the 3 week experimental period (Dai *et al.*, 1984). Control animals were given a 5% sucrose solution to drink. All drinking solutions were provided *ad libitum*. Daily intakes of fluid and morphine were determined as previously described (Dai *et al.*, 1984; Leung *et al.*, 1986a,c). The average daily intake of morphine sulphate 0.4 mg ml^{-1} solution (from day 7 onwards) was 60 ml per rat; the estimated daily intake of morphine sulphate during this period was, therefore, about 120 mg kg^{-1} . The average daily consumption of 5% sucrose solution by the control group during this period was about 62 ml per rat.

For observing recovery from the effects of chronic opiate treatment, a group of rats was first treated chronically with morphine solution for 21 days as described above. The opiate was withdrawn from the drinking water of these animals from day 22; they were given only a sucrose solution to drink for a further 2 weeks. At the end of this 2 week withdrawal period, the degree of opiate tolerance and the arterial level of catecholamines in response to sympathetic nerve excitation were examined. Control animals received sucrose solution for the whole 5 week experimental period.

Sympathetic nerve stimulation

Rats, anaesthetized with diethyl ether (BDH), were pithed through the right orbit (Gillespie *et al.*, 1970; Leung *et al.*, 1986b) and then artificially ventilated ($82 \text{ strokes min}^{-1}$, $1 \text{ ml } 100 \text{ g}^{-1}$) by a respirator (Palmer, U.K.). The pithing rod comprised two parts: an external movable 6 cm long trocar and an internal 13 cm stainless steel electrode. The whole

electrode, except for the terminal 4.5 cm, was enclosed in an insulating teflon sleeve. The exposed part of the electrode was used to stimulate the autonomic preganglionic fibres between T_1 – T_{12} in the spinal canal. The indifferent needle electrode was inserted into the subcutaneous tissues on the back of the animal, approximately opposite and parallel to the terminal end of the stimulating electrode. An abdominal laparotomy was subsequently performed and the descending aorta cannulated proximally with a heparinised polyethylene tube (internal diameter 1 mm) for blood sampling. The left jugular vein was also cannulated with a polyethylene tube (internal diameter 0.5 mm) for drug injection. After the cannulation procedures were completed, the rats were left undisturbed for 15 min before sympathetic nerve stimulation was started. Electrical impulses (70 V, 10 Hz, 0.5 ms pulse width), provided by a square-wave stimulator (Palmer, C.V.P. model), were given for 5 s. Immediately after stimulation, 6–7 ml of blood was collected from the aorta into a heparinised test tube containing 4.5 mg sodium metabisulphite (M&B) which acted as an antioxidant (Taylor and Simpkins, 1984); the collection time did not exceed 1.5 min. Contraction of skeletal muscle during electrical stimulation was reduced by i.v. treatment with gallamine triethiodide (Sigma) 5 mg kg^{-1} , expressed as the salt; the effects of possible stimulation of the parasympathetic nerves were minimised by i.p. injection of atropine sulphate (E. Merck) 1 mg kg^{-1} , expressed as the salt. Both cholinceptor blocking drugs were given 15 min before sympathetic nerve stimulation was started. The exact position of the stimulating electrode in each rat was confirmed by *post-mortem* examination. A separate group of rats was subjected to the same pithing procedure but no electrical stimulation was delivered through the pithing electrode (pithed control). All animals were killed after collection of the aortic blood samples.

Measurement of arterial catecholamines

Arterial plasma NA and Ad were extracted and purified with aluminium oxide (BDH), and measured by high performance liquid chromatography (h.p.l.c.) together with electrochemical detection (Causon *et al.*, 1981; Eriksson & Persson, 1982; Weicker *et al.*, 1984). The mobile phase was prepared as described by Eriksson & Persson (1982). The h.p.l.c. system consisted of a pump (Altex 100A) and a stainless-steel column (Waters, Bondapak C-18) which were connected to a LC-4B electrochemical detector (BAS) and a paper recorder (Kipp and Zonen BD41). Standards used were adrenaline (Codex), (–)-nor-adrenaline (Sigma) and dihydroxybenzylamine (DHBA) (Sigma). Calibration was achieved during each measurement by comparing the ratios of the

catecholamine/DHBA peak heights (Causon *et al.*, 1981; Eriksson & Persson, 1982; Weicker *et al.*, 1984).

Morphine tolerance

Development or disappearance of tolerance to the analgesic action of morphine was examined in separate groups of chronically morphine-treated rats or of post opiate-withdrawal animals by employing the tail-immersion test for analgesia, as previously described (Dai *et al.*, 1984). Twenty min after i.p. injection of morphine sulphate 2, 4, 8 or 16 mg kg⁻¹, or an equivalent volume of saline, the terminal 3 cm of the rat tails were immersed in hot water (55°C). The reaction time was taken as the period elapsing between immersion and flicking of the tails to avoid the painful stimulus. This test was performed on day 21 of the 3 week morphine treatment period or at 2 weeks following cessation of the 3 week opiate treatment.

Statistical analysis

Data are expressed as means $\pm$ s.e.mean and analysed by Student's unpaired *t* test.

Results

Chromatographic patterns of arterial catecholamines

The sequence of elution in the h.p.l.c. was NA at 5 min 30 s, Ad at 6 min 40 s and DHBA at 7 min 15 s (Figure 1). The basic chromatographic patterns of the arterial plasma samples from the acutely or chronically morphine-treated rats were similar to those of their controls.

Arterial catecholamine levels

Table 1 shows the effects of acutely administered morphine on arterial catecholamine concentrations.

Figure 1 Separation of catecholamines extracted from 2 ml of aortic plasma obtained from rats subjected to pithing procedure without (a) or with sympathetic nerve stimulation (b). $\downarrow$ = injection into port; 1 = noradrenaline; 2 = adrenaline; 3 = dihydroxybenzylamine.

In the pithed controls, treatment with morphine 16 mg kg⁻¹ did not significantly alter the basal levels of either arterial NA or Ad. Arterial concentrations of both catecholamines were significantly higher in rats subjected to sympathetic nerve stimulation. Acute morphine treatment did not influence these responses; the arterial NA or Ad concentrations were not statistically different between the saline- and morphine-treated groups.

The effects of chronic morphine treatment on arterial catecholamine levels are shown in Table 2. In the pithed controls, the values of arterial NA or Ad concentrations in sucrose- or morphine-treated rats were not significantly different. Sympathetic nerve stimulation significantly increased arterial NA and Ad in both treatment groups; however, the

Table 1 Effects of acute morphine treatment on arterial catecholamine responses to sympathetic nerve stimulation

Experimental condition	Treatment (i.p.)	Number of rats	Arterial catecholamine concentrations (ng ml ⁻¹)	
			Noradrenaline	Adrenaline
Pithed control				
Saline	1 ml kg ⁻¹	10	0.63 $\pm$ 0.09	0.43 $\pm$ 0.08
Morphine	16 mg kg ⁻¹	10	0.51 $\pm$ 0.16	0.35 $\pm$ 0.10
Sympathetic nerve stimulation				
Saline	1 ml kg ⁻¹	10	3.08 $\pm$ 0.18*	3.06 $\pm$ 0.32*
Morphine	16 mg kg ⁻¹	10	2.95 $\pm$ 0.15*	2.96 $\pm$ 0.29*

The values are the means $\pm$ s.e.mean.

* *P* < 0.001 when compared with the corresponding values of the pithed controls receiving the same treatment.

Table 2 Effects of chronic morphine treatment on arterial catecholamine responses to sympathetic nerve stimulation

Experimental condition	Drinking solution	Number of rats	Arterial catecholamine concentrations (ng ml ⁻¹)	
			Noradrenaline	Adrenaline
Pithed control	5% sucrose solution	10	0.71 ± 0.08	0.45 ± 0.14
	Morphine in 5% sucrose solution	9	0.74 ± 0.18	0.52 ± 0.08
Sympathetic nerve stimulation	5% sucrose solution	12	3.11 ± 0.21*	2.96 ± 0.30*
	Morphine in 5% sucrose solution	9	2.25 ± 0.25*†	2.45 ± 0.23*

The values are the means ± s.e.mean.

* $P < 0.001$ when compared with the corresponding values of the pithed controls of the same chronic treatment group.

† $P < 0.05$ when compared with the corresponding values of the chronic sucrose-treated controls subjected to sympathetic nerve stimulation.

arterial NA concentration in morphine-treated animals was lower ($P < 0.05$) than that in their sucrose-treated controls. The arterial Ad level in the morphine-treated group also appeared to be less than that in sucrose-treated animals, but the difference was not statistically significant.

The arterial catecholamine concentrations of rats which were formerly morphine-treated and of their sucrose-drinking controls, subjected to sympathetic nerve stimulation or similar pithing procedure but without stimulation at the end of the 2 week morphine abstinence, are shown in Table 3. All pithed controls exhibited similar basal levels of arterial NA or Ad. Sympathetic nerve stimulation increased the arterial NA and Ad concentrations in both the previously morphine-treated rats and their sucrose-

drinking controls ($P < 0.001$ for all). There were no significant differences between the arterial catecholamine levels of these two groups.

The development and disappearance of opiate tolerance

Figure 2 shows the effects of acutely administered morphine on the reaction time to pain, as determined by the tail-immersion test, at the end of the 3 week morphine-drinking period. Intraperitoneal injections of various doses of morphine sulphate (2, 4, 8 or 16 mg kg⁻¹) significantly prolonged ($P < 0.05$ for all doses) the reaction time in the sucrose-drinking controls, but not in the chronically morphine-treated rats. The reaction times of the

Table 3 Arterial catecholamine levels in previously morphine-treated rats subjected to sympathetic nerve stimulation at 2 weeks after morphine withdrawal

Experimental condition	Previously chronic treatment (drinking fluid)	Number of rats	Arterial catecholamine concentrations (ng ml ⁻¹)	
			Noradrenaline	Adrenaline
Pithed control	5% sucrose solution	8	0.68 ± 0.20	0.52 ± 0.13
	Morphine in 5% sucrose solution	9	0.51 ± 0.15	0.41 ± 0.11
Sympathetic nerve stimulation	5% sucrose solution	8	3.15 ± 0.23*	2.97 ± 0.26*
	Morphine in 5% sucrose solution	8	3.21 ± 0.19*	2.85 ± 0.25*

The values are the means ± s.e.mean.

* $P < 0.001$ when compared with the corresponding values of the pithed controls of the same previously morphine-treated group.

Figure 2 Effects of acutely administered morphine on the reaction time to pain in rats chronically treated with morphine in 5% sucrose (●, $n = 9$ for each dose) or with 5% sucrose (○, $n = 10$ for each dose) drinking solution. The values plotted are the means with s.e.mean shown by vertical bars. S = saline. * $P < 0.05$ when compared with the saline-treated control of the same group. † $P < 0.05$, †† $P < 0.001$ when compared with the corresponding values of the sucrose-drinking controls.

chronically opiate-treated animals given acute doses of morphine 8 or 16 mg kg⁻¹ were also significantly less ($P < 0.05$ and $P < 0.001$, respectively) than the corresponding values of the sucrose-drinking controls.

After 2 weeks of morphine withdrawal, i.p. injection of morphine sulphate into the previously morphine-treated rats significantly prolonged the reaction time to pain in the tail-immersion test ($P < 0.05$ and $P < 0.001$ for 8 and 16 mg kg⁻¹, respectively). The values of their reaction time were not significantly different from those of the sucrose-drinking controls (Figure 3). The patterns of their dose-response curves were also similar.

Discussion

It has been reported that the acute administration of opioids suppresses the electrical activity of sympathetic nerves in animals (Laubie *et al.*, 1977; Willette *et al.*, 1982; Jurna & Rummel, 1984). However, it was found in the present investigation that acute i.p. administration of morphine sulphate 16 mg kg⁻¹ did not significantly influence the arterial concentrations of NA or Ad in pithed controls and in rats subjected to sympathetic nerve stimulation. Assuming that the arterial levels of catecholamines reflect sympathetic activities, these findings, therefore, suggest that acute administration of this dose of morphine does not suppress electrical activity of the sympathetic nerves in pithed rats. It is unlikely that the negative findings are due to a pharmacologically ineffective amount of

Figure 3 Effects of acutely administered morphine on the reaction time to pain at 2 weeks after morphine withdrawal in 5% sucrose-drinking controls (○, $n = 8$ for each dose) or in previously 3-week morphine-treated rats (●, $n = 8$ for each dose). The values plotted are the means with s.e.mean shown by vertical bars. S = saline. * $P < 0.05$, ** $P < 0.001$ when compared with the saline-injected controls of the same group. No significant differences were found between the two groups.

injected morphine because this dose of the opiate, 16 mg kg⁻¹, produces significant analgesia in rats (Figure 2).

Chronic treatment with morphine has been reported not only to lead to the development of opiate tolerance and dependence in experimental animals but also to produce other functional changes which may not be seen after acute morphine administration (Schulz & Goldstein, 1973; Llorens *et al.*, 1978; Collier, 1980; Kromer & Steigemann, 1982; Rae & De Moraes, 1983). The present study demonstrates that the increases in arterial NA due to sympathetic nerve stimulation are significantly depressed by chronic morphine treatment. These findings support a past observation that the cardiovascular responses to sympathetic nerve stimulation are impaired in chronically morphine-treated rats (Leung *et al.*, 1986b). The present method of administering morphine in drinking fluid has been shown to be effective in inducing opiate dependence (Leung *et al.*, 1986a). As the rats had developed tolerance to the analgesic effects of acute doses, 8 or 16 mg kg⁻¹, of the opiate, after they had been drinking a morphine solution for 21 days, the current results support the idea that the occurrence of morphine tolerance is accompanied by decreased responses to sympathetic stimulation which are due to a functional change in the sympathetic system. It is conceivable that the observed phenomenon may be due to an enhanced neuronal reuptake mechanism or increased activities of monoamine oxidase or catechol-*O*-methyltransferase by chronic opiate administration; this would facilitate the clearance of catecholamine from

the circulation and subsequently lead to decreased cardiovascular changes and arterial catecholamine elevation in response to sympathetic nerve stimulation. This possibility is, however, unlikely because past findings indicate that the cardiovascular responses of chronically morphine-treated rats to acutely administered NA are not significantly different from those of naive animals (Leung *et al.*, 1986b). Direct measurement of catecholamine metabolites was carried out in a preliminary study. However, this was found to be technically difficult because the level of the major catecholamine metabolites, such as 3-methoxy-4-hydroxyphenylethylene glycol and 3-methoxy-4-hydroxymandelic acid, in the aortic plasma samples following electrical stimulation were extremely small and close to the zero baseline of the electrochemical detector recording. This finding is not at variance with the conclusions of Esler *et al.* (1984) that plasma samples from arterial blood contain extremely low amounts of catecholamine metabolites.

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