

Prevention by the 5-HT₃ receptor antagonist, ondansetron, of morphine-dependence and tolerance in the rat

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1 The effect of ondansetron, a selective 5-hydroxytryptamine₃ (5-HT₃) receptor antagonist, was studied in morphine-addicted rats. Morphine-dependence and tolerance, induced by drinking increasing concentrations of morphine sulphate in 5% sucrose solution for 3 weeks, were demonstrated by the naloxone-precipitated withdrawal syndrome and tail flick response to a thermal noxious stimulus (water at 50°C), respectively.

2 Morphine-dependence, assessed by naloxone precipitated withdrawal, was undetectable by the 6th day, when the animals drank only tap water for 7 days after the 3-week induction period.

3 When detoxified rats were offered sucrose and morphine solutions for 10 days, the recurrence of opiate solution preference with relapse to dependence and tolerance was observed.

4 Giving ondansetron (0.1 or 1 µg kg⁻¹; i.p.; twice daily) on the 14th day of, or 7 days prior to, the 3-week induction period reduced dependence and tolerance seen during the 3-week morphine induction and the 10-day drinking preference periods.

5 5-Hydroxytryptamine₂ (5-HT₂) receptor antagonism by cyproheptadine (100 or 250 µg kg⁻¹; i.p.; twice daily) did not influence morphine-dependence and tolerance.

6 These findings suggest that ondansetron may be useful for treating opiate addiction and lowering the recidivism rate.

Keywords: Ondansetron; cyproheptadine; morphine dependence; morphine tolerance

Introduction

Considerable evidence points to the involvement of the mesolimbic dopaminergic system in the actions of drugs of abuse. Dopamine has been demonstrated to play an important role in the process of reward and reinforcement and in place-preference conditioning studies. It has been shown in rats that lesioning of the nucleus accumbens, by use of kainic acid or 6-hydroxydopamine, disrupts heroin place-preference conditioning (Spyraki *et al.*, 1983), increases significantly intravenous morphine self-administration (Smith *et al.*, 1985) and reduces cocaine and heroin self-administration (Zito *et al.*, 1985). Furthermore, heroin reward has also been shown to be dependent on a dopaminergic substrate (Bozarth & Wise, 1981). The presence of 5-hydroxytryptamine₃ (5-HT₃) recognition sites in the central nervous system and their localization in areas such as the cortex, amygdala and nucleus accumbens strongly suggest that they play a role in controlling behaviour (Kilpatrick *et al.*, 1987). Recent evidence indicates that 5-HT₃ receptors are able to influence dopamine release (Blandina *et al.*, 1988), pointing to the possibility that 5-HT₃ receptor antagonists may be useful in the treatment of some aspects of drug abuse in man.

Recently, it has been reported that 5-HT₃ receptor antagonists are able to prevent the induction of place preference to nicotine or morphine (Carboni *et al.*, 1988; Koob & Goeters, 1989; Higgins *et al.*, 1992). Furthermore, ondansetron, a selective 5-HT₃ receptor antagonist (Butler *et al.*, 1988), has been shown to reduce alcohol consumption in the rat and marmoset (Oakley *et al.*, 1988; Sellers *et al.*, 1988) and to prevent the exacerbation in behavioural response to an aversive situation following withdrawal from alcohol, cocaine, nicotine or diazepam (Costall *et al.*, 1990a). The present study examines the effects of ondansetron on the morphine withdrawal syndrome and on morphine-tolerance in naive and opiate-dependent rats.

Methods

General

Male Sprague-Dawley rats (weighing 140–160 g) were used. The animals were kept in groups of 6 per cage in a rat battery. Room temperature was maintained at 22 ± 1°C and relative humidity at 60–70%. The rats were exposed to a daily cycle of 12 h light and 12 h darkness, with lights on at 06 h 00 min to 18 h 00 min. They were allowed free access to a standard balanced laboratory diet of rat pellets (Ralston Purina Co., U.S.A.) and drank either 5% sucrose (Taikoo, Hong Kong) in tap water (w/v) or morphine sulphate (Macfarlan Smith Ltd., U.K.) dissolved in a 5% sucrose solution (morphine solution). The drinking solutions were freshly prepared daily. Morphine was given in increasing concentrations of 0.1, 0.2, 0.3 and finally 0.4 mg ml⁻¹ sucrose solution at 48-h intervals; the final concentration of morphine was maintained until the end of the 3-week induction period. All animals were permitted to drink *ad libitum*.

Experimental design

Rats were randomly divided into two groups, one was given sucrose (controls) and the other received morphine; both batches were treated for 21 days. The sucrose and morphine solutions were then withdrawn for 7 days, being replaced by tap water. Following this withdrawal period, both groups of animals were allowed a free choice between drinking morphine or sucrose solutions for 10 days. During the entire experimental period (38 days), the body weight of each animal, as well as the food and fluid intakes were recorded daily. Physical dependence on morphine, as manifested by the opiate withdrawal syndrome, was evaluated for 20 min following naloxone injection (1 mg kg⁻¹, i.p.). This test for physical dependence was carried out on all rats on days 7, 10, 14, 19 and 21 after starting sucrose or morphine treatment, on days 4 and 7 during sucrose or morphine abstinence, and on day 9 of the drinking preference period. Morphine tolerance, detected by the tail flick

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reaction time to a thermal noxious stimulus (immersion in water at 50°C), was examined in all animals on the 7th, 16th and 21st day of sucrose or morphine treatment, on the 6th day of sucrose or morphine abstinence, and on the 10th day of the drinking preference period. The assessments for morphine-dependence and tolerance were performed during (a) the 3-week induction period, to determine whether the animals had developed dependence and tolerance to the opiate, (b) the 7-day withdrawal period, to find out if morphine-dependence and tolerance still persisted, and (c) the 10-day drinking preference period, to see if opiate-dependence and tolerance had recurred in these animals.

A similar regimen of tests for addiction was repeated in other groups of sucrose- or morphine-treated rats given ondansetron (Glaxo) (0.1 or 1 µg kg⁻¹, i.p.) or the 5-HT₂ receptor antagonist, cyproheptadine (Sigma) (100 or 250 µg kg⁻¹, i.p.), 2 weeks after sucrose or opiate treatment. A higher dose of ondansetron (1 µg kg⁻¹, i.p.) was also given to other groups of rats 7 days prior to sucrose or morphine treatment. All injections of ondansetron or cyproheptadine were made twice daily at 09 h 00 min and 16 h 00 min and were continued until the end of the experiment (day 38).

Evaluation of morphine-dependence

The behavioural withdrawal syndrome precipitated by naloxone was used to examine for physical dependence on morphine. Immediately after i.p. injection of naloxone (Endo) 1 mg kg⁻¹, the following behavioural parameters (withdrawal signs) were recorded for 20 min: wet-dog shakes, head shakes, diarrhoea, ptosis, chattering teeth, writhing, chewing, paw tremor and irritability to touch and handling (Collier *et al.*, 1974). The number of withdrawal signs shown by each rat was expressed as the mean of the number of signs. For comparative purposes, equivalent volumes of a solution of 0.9% NaCl in distilled water (w/v) (saline) 2 ml kg⁻¹ were given i.p. to separate groups of rats chronically treated with sucrose or morphine. The tests were carried out at 10 h 00 min on designated days throughout the entire experimental period.

Measurement of morphine tolerance

The tail-immersion test was employed to detect the development of morphine tolerance (Badawy *et al.*, 1982). This method was used to measure the antinociceptive effect of different acutely-administered doses of morphine sulphate (2, 4, 8 and 16 mg kg⁻¹, i.p.). The reaction time to nociception after

morphine injection was compared to that of the vehicle-injected control. The development of tolerance was reflected by the decrease in the antinociceptive effect of a given dose of morphine. Twenty min following i.p. injection of the opiate or saline (2 ml kg⁻¹), the terminal 3 cm of the rat tail was immersed in water at a temperature of 50°C. Reaction time was expressed as the time elapsing between immersion of the tail and when it flicked out of the water.

Drugs

Ondansetron hydrochloride dihydrate (Glaxo), cyproheptadine hydrochloride (Sigma) and naloxone hydrochloride (Endo) were freshly prepared in saline; the doses are expressed as bases.

Statistical analysis

The results are expressed as means ± s.e.mean. Data were analysed by the two-tailed Student's *t* test.

Results

General observations

During the 3-week induction period, the body weights of the sucrose- or morphine-treated rats increased steadily during the first 15 days with no significant difference between the two treatments (sucrose-treated 160 ± 5.2–265 ± 3.4 g; morphine-treated 171.8 ± 2.3–260 ± 2.5 g) (weights on the 1st and 15th day). Measurements taken on days 16 to 21 indicated that the body weights of the morphine-treated rats were slightly but significantly less ($P < 0.05$ – $P < 0.001$) than those of the sucrose-treated group (sucrose-treated 310 ± 1.5–322 ± 2.1 g; morphine-treated 270 ± 2.5–285 ± 2.8 g) (weights on the 16th and 21st day). The daily fluid intake of morphine (38.2 ± 5.2–48.9 ± 7.1 ml/rat/day) was similar to the intake of sucrose (47.4 ± 5.3–51.8 ± 4.5 ml/rat/day) throughout the 3-week period.

Production and recurrence of morphine-dependence and tolerance in sucrose- or morphine-treated rats

During the 21-day induction period, i.p. injection of naloxone 1 mg kg⁻¹ precipitated marked withdrawal effects in rats chronically treated with morphine in their drinking fluid. This

Table 1 Effect of ondansetron on the naloxone-precipitated withdrawal syndrome in sucrose- or morphine-treated rats

| Days of treatment | Sucrose treatment | Morphine treatment | Sucrose treatment + ^a ondansetron i.p. twice daily | | Morphine treatment ^a ondansetron i.p. twice daily | | ^b ondansetron i.p. twice daily (1 µg kg ⁻¹) | ^b ondansetron i.p. twice daily (1 µg kg ⁻¹) |
|---|-------------------|----------------------------|--|--------------------------|---|--------------------------|--|--|
| | | | (0.1 µg kg ⁻¹) | (1 µg kg ⁻¹) | (0.1 µg kg ⁻¹) | (1 µg kg ⁻¹) | | |
| A. Morphine/Sucrose | | | | | | | | |
| 7 | 0.33 ± 0.14 | 2.08 ± 0.31 ⁺ | 0.00 ± 0.00 | 0.50 ± 0.22 | 1.33 ± 0.08 | 1.50 ± 0.50 | 0.31 ± 0.00 | 0.00 ± 0.00* |
| 10 | 1.00 ± 0.33 | 3.32 ± 0.82 ⁺ | 0.17 ± 0.17 | 0.67 ± 0.21 | 2.83 ± 0.09 | 2.17 ± 0.40 | 1.30 ± 0.48 | 1.17 ± 0.48* |
| 14 | 0.25 ± 0.13 | 3.75 ± 0.41 ⁺⁺ | 0.00 ± 0.00 | 0.50 ± 0.22 | 3.33 ± 0.71 | 3.67 ± 0.30 | 0.40 ± 0.15 | 1.21 ± 0.50** |
| 19 | 0.42 ± 0.15 | 4.08 ± 0.29 ⁺⁺⁺ | 0.30 ± 0.21 | 0.50 ± 0.20 | 1.90 ± 0.04* | 2.17 ± 0.60* | 0.31 ± 0.12 | 1.30 ± 0.41** |
| 21 | 0.67 ± 0.21 | 4.67 ± 0.36 ⁺⁺⁺ | 0.57 ± 0.26 | 0.47 ± 0.17 | 1.24 ± 0.47** | 1.83 ± 0.50** | 0.21 ± 0.15 | 1.00 ± 0.10*** |
| B. Day After sucrose morphine abstinence | | | | | | | | |
| 4 | 0.67 ± 0.19 | 1.00 ± 0.25 | 0.20 ± 0.08 | 0.27 ± 0.10 | 0.50 ± 0.22 | 0.50 ± 0.20 | 0.22 ± 0.10 | 0.61 ± 0.14 |
| 7 | 0.50 ± 0.23 | 0.67 ± 0.22 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.33 ± 0.20 | 0.37 ± 0.05 | 0.21 ± 0.10 | 0.23 ± 0.11 |
| C. Day of drinking-preference period | | | | | | | | |
| 9 | 0.42 ± 0.13 | 4.85 ± 0.32 ⁺⁺⁺ | 0.37 ± 0.11 | 0.64 ± 0.20 | 0.57 ± 0.23*** | 0.42 ± 0.15*** | 0.67 ± 0.33 | 0.83 ± 0.31** |

Values are expressed as mean ± s.e.mean ($n = 9-24$) indicating mean numbers of naloxone-precipitated withdrawal signs. ^aRepresents commencement of ondansetron administration from the 14th day of sucrose or morphine treatment. ^bRepresents commencement of ondansetron administration 7 days prior to sucrose or morphine treatment. ⁺ $P < 0.05$, ⁺⁺ $P < 0.01$, ⁺⁺⁺ $P < 0.001$, compared to the sucrose-treated controls. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared to the morphine-treated controls.

group of animals showed a significantly larger number of withdrawal signs when compared to the sucrose-treated rats injected with naloxone (Table 1) from the 7th day, and these signs persisted until the 21st day of treatment with increasing severity (2 fold difference from day 7), indicating that these animals were already physically dependent on the opiate at the end of the 1st week. During the 7-day withdrawal period of sucrose or morphine, injection of naloxone on days 4 and 7 failed to produce significant withdrawal effects (Table 1); this suggests that these rats, previously treated chronically with morphine, showed no significant signs of opiate dependence from day 4 of its withdrawal. However, during the 10-day drinking preference period, challenge with naloxone on the 9th day in the previously morphine-treated rats elicited a significantly larger number of withdrawal signs when compared to their previously sucrose-treated controls (Table 1), indicating that physical dependence on the opiate had recurred in these animals.

Morphine tolerance, as manifested by the reaction time to noxious stimulus, using the tail-immersion test, is shown in Figure 1. Intraperitoneal injection of morphine sulphate (2–16 mg kg⁻¹) dose-dependently increased the reaction time in the sucrose-treated controls. The morphine-treated animals, however, did not show any significant differences from the sucrose-treated controls when measured on the 7th day of the opiate treatment period, indicating that morphine tolerance had not yet developed (Figure 1a). However, the reaction time of opiate-drinking rats to the higher doses of morphine (4–16 mg kg⁻¹, i.p.) when measured on days 16 and 21 of the 3-week induction period, appeared to be no different from that

of their own morphine-consuming controls injected with saline. Instead, the reaction time was significantly less than that of the sucrose-treated rats (Figure 1b, c), suggesting that the chronically morphine-treated animals had already developed tolerance to the antinociceptive action of acutely administered morphine from day 16 onwards.

The reaction time in the tail-immersion test on the 6th day after withdrawal of sucrose or morphine treatment was similar in the sucrose- and morphine-treated rats, implying that the animals had been successfully detoxified by the 7-day withdrawal period (Figure 1d). When the animals were tested on the last day of the 10-day drinking preference period, rats previously consuming morphine solutions did not show significantly different responses to the various doses of acutely administered morphine compared to their own morphine-consuming controls administered saline (Figure 1e); this indicates that previously morphine-treated animals had again developed tolerance to the opiate.

Effect of ondansetron on morphine-dependence during 21-day sucrose or morphine treatment

Ondansetron (0.1 or 1 µg kg⁻¹, given i.p.; twice daily), commencing either 7 days prior to, or 14 days after, starting sucrose treatment, did not have any significant effect on naloxone-precipitated withdrawal signs when compared to their sucrose-control values (Table 1) during the period of 21-day treatment, 7-day sucrose withdrawal, or 10-day drinking preference.

Ondansetron (0.1 or 1 µg kg⁻¹, i.p.; twice daily), given from day 14 of morphine treatment, significantly reduced the naloxone-precipitated withdrawal effects measured on days 19 and 21 of the 21-day opiate treatment regimen and on day 9 of the 10-day drinking preference period (Table 1). When ondansetron (1 µg kg⁻¹) treatment was started 7 days before the morphine regimen, there was a significant reduction in the naloxone withdrawal syndrome from the 7th day, and this persisted until the 21st day of opiate treatment (Table 1), and also on the 9th day of the 10-day drinking preference period. The administration of ondansetron starting either 7 days before, or 14 days after, starting morphine treatment, however, did not significantly influence naloxone-induced withdrawal signs measured during the 7-day morphine withdrawal period (Table 1).

Effect of ondansetron on morphine tolerance during 21-day sucrose or morphine treatment

Ondansetron (0.1 or 1 µg kg⁻¹) administered in both regimens did not have any significant effect on the tail flick reaction time in response to acutely administered morphine in sucrose-treated rats tested on the 21st day of sucrose treatment (Figure 2a), except for the lower dose of ondansetron (0.1 µg kg⁻¹), commencing on day 14 of sucrose treatment, which significantly increased the reaction time following morphine injection (4 mg kg⁻¹) (Figure 2a).

Morphine-treated rats showed no significant differences in responses to the various doses of acutely administered morphine, but their reaction time was significantly shorter than that of the sucrose-treated controls (Figure 2b), indicating that tolerance to the opiate had developed in these chronically morphine-treated animals. Administration of ondansetron (0.1 or 1 µg kg⁻¹), starting on the 14th day of morphine treatment, significantly lengthened the reaction time in response to the higher doses of acutely administered morphine (8 or 16 mg kg⁻¹) in opiate-treated animals suggesting that morphine tolerance was antagonized (Figure 2b). The higher dose of ondansetron (1 µg kg⁻¹), starting 7 days before morphine treatment, lengthened further the reaction time to nociception but the values were not significantly different from those of the sucrose-treated controls (Figure 2b).

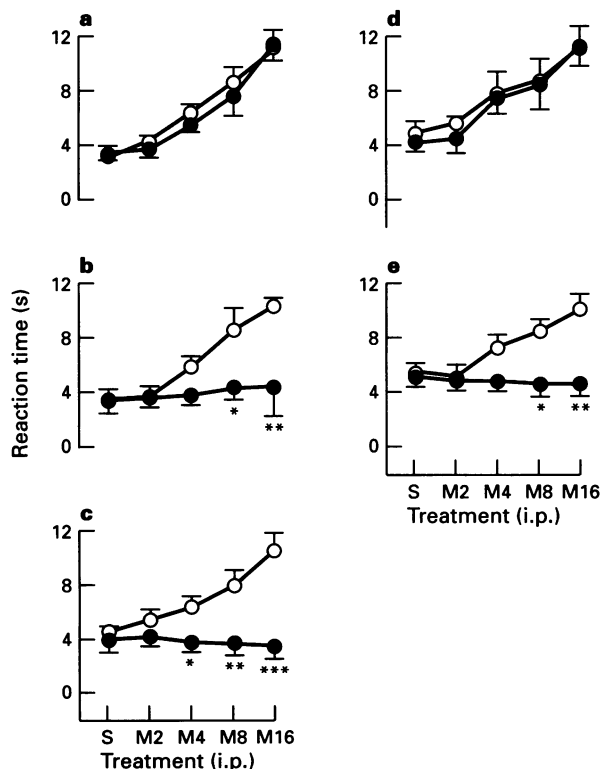


Figure 1 Effect of acutely administered morphine on the reaction time of sucrose-treated (○) and morphine-treated (●) rats measured by the tail-immersion test. (a), (b) and (c) show the data obtained on days 7, 16 and 21 of sucrose or morphine treatment, respectively; (d) shows the data obtained on day 6 after sucrose or morphine withdrawal; (e) shows the data obtained on day 10 of the 10-day drinking preference period. S, saline (2 ml kg⁻¹, i.p.); M, morphine (mg kg⁻¹, i.p.) Values are means ± s.e. of 6–9 rats for each dose. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, compared to the corresponding sucrose-treated controls.

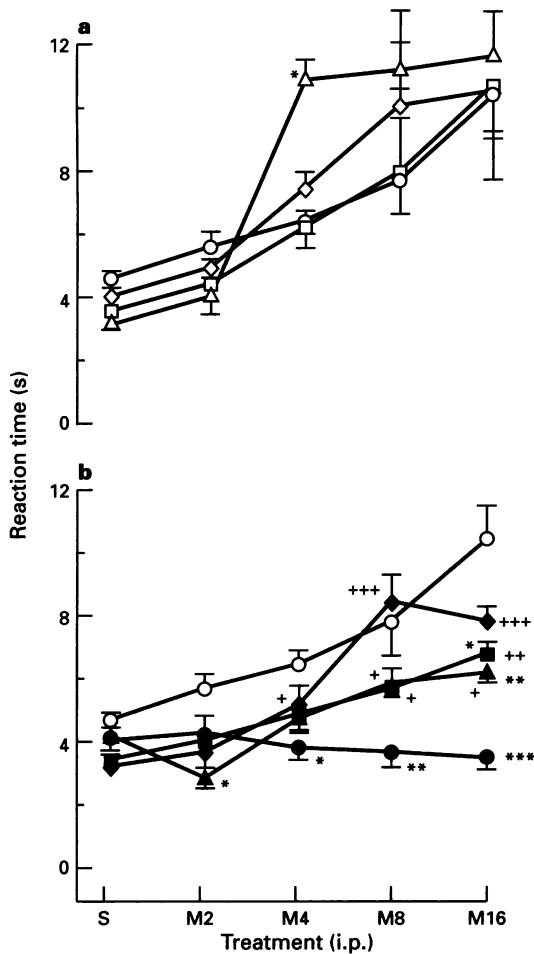


Figure 2 Effect of ondansetron on the tail flick reaction time in response to acutely administered morphine on the 21st day of sucrose or morphine treatment in (a) sucrose-treated and (b) morphine-treated rats. (a) (○) Sucrose-treated controls; (△) sucrose + ondansetron $0.1 \mu\text{g kg}^{-1}$, i.p. twice daily and (□) sucrose + ondansetron $1 \mu\text{g kg}^{-1}$, i.p. twice daily given from the 14th day of sucrose administration; (◇) sucrose + ondansetron $1 \mu\text{g kg}^{-1}$, i.p. twice daily given 7 days prior to sucrose treatment. (b) (●) Morphine-treated controls; (▲) and (■) morphine + ondansetron 0.1 and $1 \mu\text{g kg}^{-1}$, i.p. twice daily given from the 14th day of morphine administration, respectively; (◆) morphine + ondansetron $1 \mu\text{g kg}^{-1}$, i.p. twice daily given 7 days prior to morphine treatment. S, saline (2 ml kg^{-1} , i.p.); M, morphine (mg kg^{-1} , i.p.). Values are expressed as the means \pm s.e.mean of 6–9 rats in each group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared to the corresponding sucrose-treated controls. + $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.001$, compared to the corresponding morphine-treated controls.

Effect of ondansetron on morphine tolerance in sucrose- or morphine-treated rats during the 10-day drinking preference period

Ondansetron, administered either 7 days prior to, or 14 days after, starting sucrose treatment, did not have any significant effect on the reaction time to acutely administered morphine in sucrose-treated rats (Figure 3a) when measured on the 10th day of the drinking preference period.

The lengthened reaction time following acutely administered morphine, seen in previously sucrose-treated rats tested on the 10th day of the drinking preference period, was significantly absent in those which had been previously morphine-treated, indicating recurrence of morphine tolerance in these animals (Figure 3b). Ondansetron, administered in both regimens, significantly restored the increased reaction time induced by the acute opiate dose. The values in reaction time among these groups were not significantly different from those of the previously sucrose-treated controls (Figure 3b).

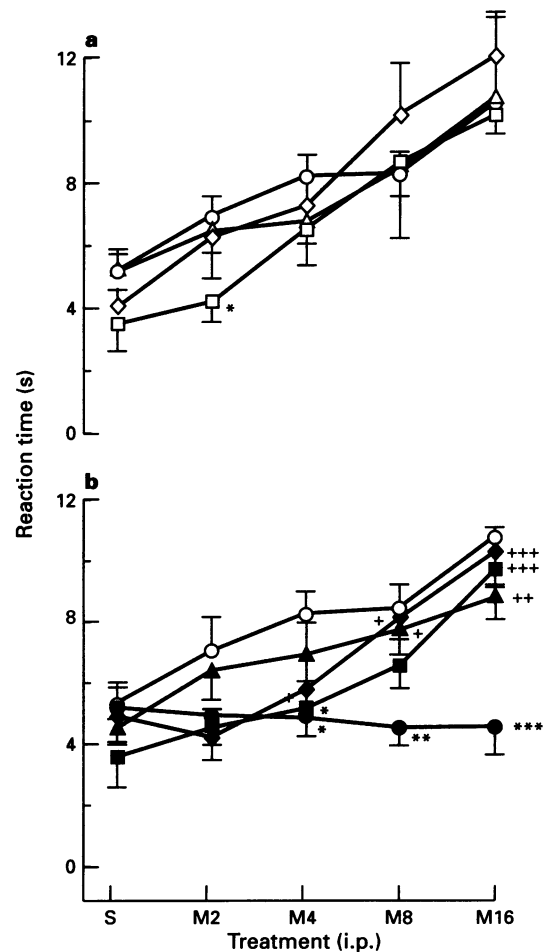


Figure 3 Effect of ondansetron on the tail flick reaction time in response to acutely administered morphine on the 10th day of the drinking preference period in (a) previously sucrose-treated and (b) previously morphine-treated rats. (○) Previously sucrose-treated controls; (△) and (□) sucrose + ondansetron $0.1 \mu\text{g kg}^{-1}$ and $1 \mu\text{g kg}^{-1}$, i.p. twice daily given from the 14th day of sucrose administration, respectively; (◇) sucrose + ondansetron $1 \mu\text{g kg}^{-1}$, i.p. twice daily given 7 days prior to sucrose treatment. (●) Previously morphine-treated controls; (▲) and (■) morphine + ondansetron $0.1 \mu\text{g kg}^{-1}$ and $1 \mu\text{g kg}^{-1}$, i.p. twice daily given from the 14th day of morphine administration, respectively; (◆) morphine + ondansetron $1 \mu\text{g kg}^{-1}$, i.p. twice daily given 7 days prior to morphine treatment. S, saline (2 ml kg^{-1} , i.p.); M, morphine (mg kg^{-1} , i.p.). Values are means \pm s.e.mean of 6–9 rats in each group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$, compared to the previously sucrose-treated controls. + $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.001$, compared to the previously morphine-treated controls.

Effect of cyproheptadine on morphine-dependence and morphine-tolerance in sucrose- or morphine-treated rats

The 5-HT₂ receptor antagonist, cyproheptadine, when administered at 100 or $250 \mu\text{g kg}^{-1}$ i.p., twice daily 14 days after starting sucrose treatment, did not change significantly the naloxone-precipitated withdrawal syndrome in sucrose-treated rats during the 21-day sucrose treatment period, or sucrose abstinence, or the 10-day drinking preference period (sucrose 0.17 ± 0.03 – 1.00 ± 0.3 ; cyproheptadine $100 \mu\text{g kg}^{-1}$ + sucrose 0.0 ± 0.0 – 0.67 ± 0.21 ; cyproheptadine $250 \mu\text{g kg}^{-1}$ + sucrose 0.0 ± 0.0 – 0.67 ± 0.23). Similarly, both doses of cyproheptadine did not have significant effects on the naloxone-precipitated withdrawal signs in morphine-treated rats except on day 21 of morphine treatment and on day 9 of the drinking preference period (Table 2).

Cyproheptadine, given in a similar regimen to the morphine-treated rats, did not have a significant action on the tail flick reaction time in response to different doses of acutely

Table 2 Effect of cyproheptadine on the naloxone-precipitated withdrawal syndrome in morphine-treated rats

| Day of treatment | Morphine treatment | Morphine treatment + ^a cyproheptadine i.p. twice daily | |
|---|--------------------|---|----------------------------|
| | | (100 µg kg ⁻¹) | (250 µg kg ⁻¹) |
| A. Morphine | | | |
| 7 | 2.08 ± 0.31 | 1.75 ± 0.40 | 2.20 ± 0.40 |
| 10 | 3.32 ± 0.82 | 2.83 ± 0.40 | 2.83 ± 0.10 |
| 14 | 3.75 ± 0.41 | 3.33 ± 0.49 | 3.17 ± 0.48 |
| 19 | 4.08 ± 0.29 | 3.03 ± 0.21 | 2.20 ± 0.66* |
| 21 | 4.67 ± 0.36 | 2.33 ± 0.42* | 3.67 ± 0.44 |
| B. Day After morphine abstinence | | | |
| 4 | 1.00 ± 0.25 | 0.47 ± 0.10 | 0.33 ± 0.21 |
| 7 | 0.67 ± 0.22 | 0.30 ± 0.05 | 0.22 ± 0.14 |
| C. Day of drinking-preference period | | | |
| 9 | 4.50 ± 0.29 | 2.50 ± 0.34* | 4.00 ± 0.37 |

Values are expressed as means ± s.e. mean of 6–12 rats in each group. ^aRepresents commencement of cyproheptadine administration from the 14th day of morphine treatment. **P* < 0.05, compared to the morphine-treated controls.

administered morphine (2–16 mg kg⁻¹, i.p.), except on day 21 of morphine treatment and day 10 of the drinking preference period during which the reaction time in response to the highest dose of morphine (18 mg kg⁻¹) was significantly increased by the bigger dose of cyproheptadine (250 µg kg⁻¹) (Table 3). Again, cyproheptadine had no significant effect on morphine tolerance in sucrose-treated animals (data not shown).

Discussion

It has been demonstrated that a gradual increase of morphine concentration in sucrose drinking water can produce physical dependence, and a relapse after successful opiate withdrawal and detoxification (Dai *et al.*, 1984). In the present study, using a similar dose regimen, physical dependence on morphine, as shown by the occurrence of naloxone-precipitated withdrawal symptoms and the development of tolerance to the analgesic effect of an acutely-administered dose of morphine, was established over a 21-day period. Physical dependence was evident from the 14th day of chronic morphine treatment and was absent following a 7-day opiate withdrawal period; however, physical dependence was regained when the detoxified rats

were exposed to a 1-day free choice situation when they had access to both sucrose and morphine solutions.

The recurrence of morphine addiction after the withdrawal period was further supported by the observation that detoxified rats showed a clear preference for morphine solution (Hui *et al.*, 1993). In this study, in order to investigate whether ondansetron, a selective 5-HT₃ receptor antagonist (Butler *et al.*, 1988), was able to influence morphine-dependence and tolerance in naive rats and in animals which had already been addicted and shown to have dependence and tolerance to the opiate, ondansetron was administered either 7 days prior to, or 14 days after, starting sucrose or morphine treatment.

The findings provide clear evidence that ondansetron, when administered prior to and during morphine administration, significantly prevents morphine-dependence and tolerance. The effects of these treatments were also evident after discontinuing the drug regimens. Thus, in a free choice situation, previously morphine-dependent animals which had received ondansetron showed no significant differences in naloxone-precipitated withdrawal signs and reaction time to acutely-administered morphine when compared to their sucrose-treated controls. Ondansetron itself did not induce dependence or tolerance to the opiate. Bradley *et al.* (1986) have identified the pharmacological characteristics of the 5-HT₃ receptor site, and have indicated that one of the criteria is that this receptor should be blocked selectively by low concentrations of MDL 72222, ICS 205-930, granisetron or ondansetron (Bradley *et al.*, 1986). In the current study, relatively low doses of ondansetron effectively antagonised morphine-dependence and tolerance. Indeed, the two concentrations (0.1 and 1 µg kg⁻¹) of ondansetron used in this study have been demonstrated by other researchers to be effective in inhibiting the increased behavioural suppression following withdrawal from treatment with diazepam and other drugs of abuse (Costall *et al.*, 1989; 1990a) and in reducing raised mesolimbic dopaminergic activity in the rat and marmoset (Costall *et al.*, 1987). These concentrations are also within the effective range for antagonizing the 5-HT₃-mediated bradycardia (von Bezold-Jarisch reflex), a reliable *in vivo* assay of 5-HT₃ receptor antagonist activity (Butler *et al.*, 1988). Based on the foregoing, the observed effects of ondansetron can therefore be reasonably attributed to 5-HT₃ antagonism.

The involvement of the 5-HT₃ receptor in inhibiting the behavioural changes following withdrawal from drugs of abuse has been documented. Using the light/dark exploration test in the mouse and social interaction test in the rat, Costall *et al.* (1990a) showed that peripheral administration of ondansetron during the period of ethanol, nicotine or cocaine withdrawal, prevents the exacerbation in suppressed behaviour following

Table 3 Effect of cyproheptadine on the tail flick reaction time (s) in response to acutely administered morphine in morphine-treated rats

| Treatment | Saline (2 ml kg ⁻¹ i.p.) | Acute morphine (mg kg ⁻¹ , i.p.) | | | |
|---|-------------------------------------|--|-------------|-------------|--------------|
| | | 2 | 4 | 8 | 16 |
| A. Day 21 after morphine treatment | | | | | |
| Morphine only | 3.05 ± 0.70 | 3.70 ± 0.18 | 4.42 ± 0.10 | 3.80 ± 0.35 | 3.72 ± 0.34 |
| Morphine + ^a Cyproheptadine 100 µg kg ⁻¹ | 2.72 ± 0.15 | 3.68 ± 0.06 | 4.26 ± 0.30 | 3.44 ± 0.29 | 4.18 ± 0.22 |
| Morphine + ^a Cyproheptadine 250 µg kg ⁻¹ | 3.36 ± 0.25 | 4.03 ± 0.53 | 3.98 ± 0.35 | 4.00 ± 1.50 | 7.70 ± 1.84* |
| B. Day 10 of drinking-preference period | | | | | |
| Morphine only | 5.58 ± 0.65 | 5.83 ± 0.52 | 4.92 ± 0.48 | 4.60 ± 0.50 | 4.56 ± 0.80 |
| Morphine + ^a Cyproheptadine 100 µg kg ⁻¹ | 3.93 ± 0.18 | 4.60 ± 0.54 | 4.40 ± 0.74 | 3.84 ± 0.36 | 3.55 ± 0.32 |
| Morphine + ^a Cyproheptadine 250 µg kg ⁻¹ | 3.86 ± 0.48 | 4.17 ± 0.22 | 5.15 ± 0.34 | 4.50 ± 0.37 | 7.50 ± 2.07* |

Values are expressed as means ± s.e. mean of 12–24 rats in each group. ^aRepresents commencement of cyproheptadine administration from the 14th day of the morphine treatment. **P* < 0.05, compared to the corresponding morphine-treated control.

withdrawal from these drugs of abuse. Furthermore, it has also been demonstrated that ondansetron is highly effective in preventing behavioural suppression and weight loss following withdrawal from sub-chronic treatment with anxiolytic agents (Costall *et al.*, 1989). One possible explanation for the inhibitory effect of ondansetron on morphine-tolerance and dependence may be due to the manifestation of the anxiolytic properties of ondansetron. However, as buspirone, an anti-anxiety agent, fails to prevent the development of withdrawal following chronic treatment with diazepam (Costall *et al.*, 1989), this would exclude such a possibility.

Ondansetron may, therefore, inhibit craving for drugs of abuse, by influencing a fundamental process underlying drug-dependence. The ventral tegmental area has an important role in mediating the rewarding effects of drugs of abuse (Bozarth, 1987) and overactivity of the system can be inhibited by 5-HT₃ receptor antagonists (Costall *et al.*, 1987; Hagan *et al.*, 1987). It is, thus, likely that the influence of ondansetron is on the dopaminergic projections from the ventral tegmental area. Activation of the mesolimbic dopaminergic system is considered to play an important role in conveying the rewarding effect elicited by drugs of abuse (Di Chiara & Imperato, 1988). It has been speculated that the suppression of dopamine output in the limbic forebrain may correlate with the aversive symptomatology experienced during withdrawal from drugs of abuse. Indeed, experiments in other laboratories have shown that withdrawal from chronic administration of ethanol, morphine, cocaine or amphetamine is associated with a marked reduction in extracellular dopamine concentration in the ventral striatum, as measured by microdialysis (Rosetti *et*

al., 1992). It has also been shown that the amygdala and dorsal raphe nucleus may be the important loci of action for ondansetron to mediate the reduction in aversive behaviour caused by withdrawal from drugs of abuse (Costall *et al.*, 1990b).

The mechanism by which ondansetron prevents morphine-dependence has not been investigated in this study. It is possible that the 5-HT₃ receptor antagonist exerts its effect by modulating the mesolimbic dopaminergic mechanism via an action on the 5-HT₃ receptors. The mechanism for the prevention by ondansetron of tolerance to the antinociceptive effect of morphine, however, is likely to be related to a peripheral action. This is reinforced by the tail flick assay employed in this study which represents a bulbospinal reflex and therefore suggests that any interaction between ondansetron and morphine, with regard to antinociception, must be at the spinal and not supraspinal level. This interaction is also likely to be independent of the spinal 5-HT₃-mediated antinociception *per se* since the latter cannot be overcome by naloxone pretreatment (Alhaider *et al.*, 1991).

The data from the present study point to the reasonable conclusion that ondansetron, through 5-HT₃ receptor antagonism, may be useful in the treatment of opiate addiction, especially in reducing the relapse rate after detoxification.

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