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Effects of ondansetron administration [1] on opioid withdrawal syndrome observed in rats

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Abstract

This study tested whether a 5-HT_3 receptor antagonist could reverse the signs of precipitated opioid withdrawal. Rats were treated with either saline or morphine for 4 days. After the four days, half of the rats in each group received naloxone and half received saline. Each animal also received one of four doses of ondansetron (0, 1, 2 and 4 mg/kg i.p.). Administration of ondansetron to rats receiving naloxone after chronic morphine decreased the intensity of withdrawal signs such as increased defecation, jumping and wet-dog shakes, elevated the nociceptive threshold values which were decreased by precipitated withdrawal, but produced no change in urination, rectal temperature or salivation. The effects exhibited by ondansetron administration may be explained through interference of its 5-HT_3 receptor antagonist activity with serotoninergic mechanisms involved in the regulation of these withdrawal symptoms. The use of this drug is thus suggested as a possible treatment of opioid withdrawal signs in heroin addicts. © 1997 Published by Elsevier Science B.V.

Keywords: Opioid withdrawal syndrome; (Rat); Ondansetron; Withdrawal

1. Introduction

Acute and chronic morphine administration to rats modifies serotonin metabolism and 5-HT receptor expression (Martin and Jasinski, 1977; Redmond and Krystal, 1984; Bhargava, 1994). Furthermore, the neurotransmitter serotonin has been demonstrated to be involved in the manifestation of some morphine withdrawal signs. In rodents, serotonin has been reported to affect wet-dog shake behaviour (Kruszewska and Langwinski, 1983) and to be associated with abstinence jumping (Samanin et al., 1980a,b; Van der Laan and Hillen, 1986). Additionally a role of serotonin has been evidenced in other withdrawal signs such as diarrhoea (Samanin et al., 1980a; Beubler et al., 1984) or hypothermia (Neal and Sparber, 1986b; Gulati and Bhargava, 1990). A reduction in the number of 5-HT receptors in the brain-stem of morphine-dependent rats has been reported (Samanin et al., 1980a). Furthermore during opioid abstinence a modified 5-HT subtype receptor expression is described in rats: 5-HT_1 receptors appear to be up-regulated in certain brain regions; 5-HT_{1A} receptors are down expressed in the hypothalamus but 5-HT_2 receptors are not affected (Gulati and Bhargava, 1988, 1989, 1990).

Several authors have attempted to control the morphine withdrawal syndrome by using drugs capable of recognising the 5-HT receptors (Zifa and Fillon, 1992). The administration of 5-HT receptor agonists or antagonists appears to affect certain components of the opioid withdrawal syndrome. The administration of buspirone, a 5-HT_1 receptor agonist, has been reported to attenuate opiate abstinence behaviour symptoms in rhesus monkeys (Aceto and Bowman, 1993). In particular, agents reported to have central serotoninergic agonist properties such as quipazine and *meta*-chlorophenylpiperazine (Samanin et al., 1980a), or a 5-HT_2 receptor antagonist, mianserine (Neal and Sparber, 1986a), block the naloxone-induced jumping in morphine-dependent rats. Furthermore, cyproheptadine, a 5-HT_2 receptor antagonist, reduces jumping precipitated by naloxone in morphine-treated rats (Cervo et al., 1981). Drugs exhibiting mixed 5-HT_1 and 5-HT_2 receptor antagonist activity, like methergoline, decrease jumping (Samanin et al., 1980b) or wet-dog shakes (Kruszewska and Langwinski, 1983) in morphine-dependent rats treated with naloxone or methisergide, and attenuate jumping and wet-dog shakes (El-Kadi and Sharif, 1995) in opioid-dependent

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[1] Zofran is the trade name for ondansetron (injectable solution) manufactured by Glaxo SpA, Verona, Italy.

mice injected with naloxone. The 5-HT_1 receptor agonist, hydroxy-dipropyl-amino-tetraline (DPAT), induces hypothermia in abstinent rats (Gulati and Bhargava, 1990).

In addition the 5-HT_2 receptor antagonists, ketanserin or pirenperone, have been reported to counteract the hypothermia induced by naloxone in morphine-treated rats (Neal and Sparber, 1986b) while the 5-HT_1 and 5-HT_2 receptor antagonist, cyproheptadine, aggravates hypothermia in dependent mice withdrawn from morphine (El-Kadi and Sharif, 1995). Additionally the 5-HT_1 receptor agonist, *meta*-chlorophenylpiperazine (Samanin et al., 1980a) and the 5-HT_2 receptor antagonist, ketanserin (Beubler et al., 1984), have been reported to block withdrawal diarrhoea in rats.

These findings suggest that serotonin is involved in the production of some withdrawal symptoms and also that several different 5-HT receptor agonists or antagonists decrease naloxone-precipitated withdrawal signs. There is thus a rational basis for seeking and using other 5-HT receptor-affecting agents for the control of opioid abstinence symptomatology. Since the autonomic signs appear to be a more sensitive index of the opioid withdrawal syndrome than are behavioural signs (Buccafusco et al., 1984), it is important to select an agent capable of interfering with behavioural, autonomic and somatosensitive signs. In particular, 5-HT_3 receptor antagonists have been reported to interact with 5-HT_3 receptors abundantly located on central nervous system dopaminergic neurones (Montgomery et al., 1993). Dopamine release in the nucleus accumbens stimulated by morphine is thus inhibited and the related behaviour is interfered with (Costall et al., 1987; Pei et al., 1993). Specifically the 5-HT_3 receptor antagonist, ondansetron, reduces morphine self-administration in rats (Borg and Taylor, 1994) and modifies the morphine place preference conditioning (Acquas et al., 1988; Higgins et al., 1992).

Furthermore in animals and human subjects, ondansetron has been reported to interfere with motility of the gastrointestinal tract (Bradley et al., 1986; Talley et al., 1990; Lamers, 1991), and urinary bladder (Corsi et al., 1991) as well as the pain threshold levels (Giordano and Dyche, 1989; Sufka et al., 1992). Ondansetron was thus a possible candidate for controlling several deprivation signs and for the study of its effects on withdrawal symptoms such as altered faeces and urine excretion values, temperature and pain threshold levels, salivary, jumping and wet-dog shake behaviour.

We now report on the activities exerted by ondansetron on naloxone-precipitated withdrawal in morphine-dependent rats.

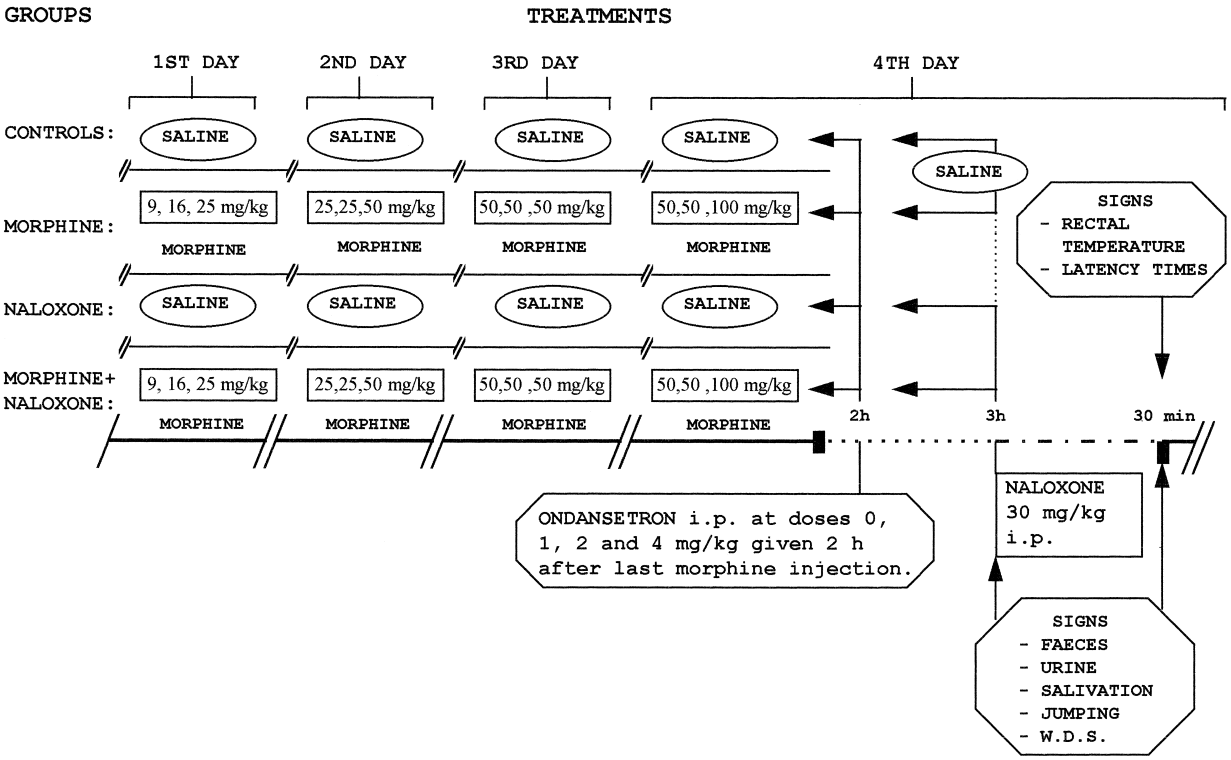

Fig. 1. Scheme illustrating the treatment with saline, morphine, naloxone, morphine plus naloxone and ondansetron: withdrawal signs observed 30 min after naloxone injection (rectal temperature, latency times), or for 30 min after naloxone injection (excretion of faeces, urine, saliva, jumping and wet-dog shakes (w.d.s.)).

2. Materials and methods

2.1. General procedures

Male Sprague–Dawley rats (Charles River, Calco) weighing 200 ± 10 g were used in the experiments. The scheme of treatments with saline, morphine, naloxone, morphine plus naloxone and ondansetron is described in Fig. 1. The experiments were performed with four groups of 32 animals each: two groups received chronic saline and the other two received chronic morphine. Precipitated withdrawal was induced in one saline and one morphine group by injection of naloxone. The other two groups, which acted as controls, received a saline injection. Morphine was administered intraperitoneally (three injections each day at 150-min intervals) for four days, in doses of 9, 16 and 25 mg/kg (1st day); 25, 25 and 50 mg/kg (2nd day), 50, 50 and 50 mg/kg (3rd day); 50, 50 and 100 mg/kg (4th day). Naloxone was given in a dose of 30 mg/kg, i.p. 180 min after the last morphine injection only on the last day. 2 h after the last morphine injection, 8 animals in each of the four groups received one of the four doses of ondansetron (0, 1, 2 and 4 mg/kg). At the beginning of the experiment the morphine group was larger since previous experience had shown that morphine treatment caused about 30% mortality. Animals were selected at random for the treatment with ondansetron.

2.2. Behavioural testing

The animals were placed in plastic cylinders (50 × 18 cm) over previously weighed filter paper dishes. The following signs were observed: faeces and urine excretion, rectal temperature and latency time values, salivation, jumping and wet-dog shake behaviour.

The signs were evaluated in the following way:

– Faeces excretion by weighing the stools on the paper dishes.

– Urine excretion by weighing the fluid content absorbed on the paper dishes after faeces removal.

– Rectal temperature with a thermal probe.

– Latency times with the tail-flick technique (Harris et al., 1969), using equipment provided by Socrel, Milan (Italy). The technique is based on measurement of the time (s) between exposure of the tail to thermal irradiation (110°C) and flicking of the tail (tail-flick latency). A 20-s cut-off time was applied.

– Salivation.

– Jumping.

– Wet-dog shakes.

These three signs, hypersalivation, jumping and wet-dog shakes appeared only in groups receiving morphine plus naloxone and four doses of ondansetron (0, 1, 2 and 4 mg/kg) and were evaluated by scoring the intensity of the sign from 0 to 3 points (hypersalivation), by counting the number of events (jumping and wet-dog shakes) and by counting the number of animals exhibiting modified behaviours.

Excretion of faeces and urine, salivation, jumping and wet-dog shakes were observed for 30 min after naloxone injection. Body temperature and pain threshold levels were evaluated at the 30th min after naloxone injection.

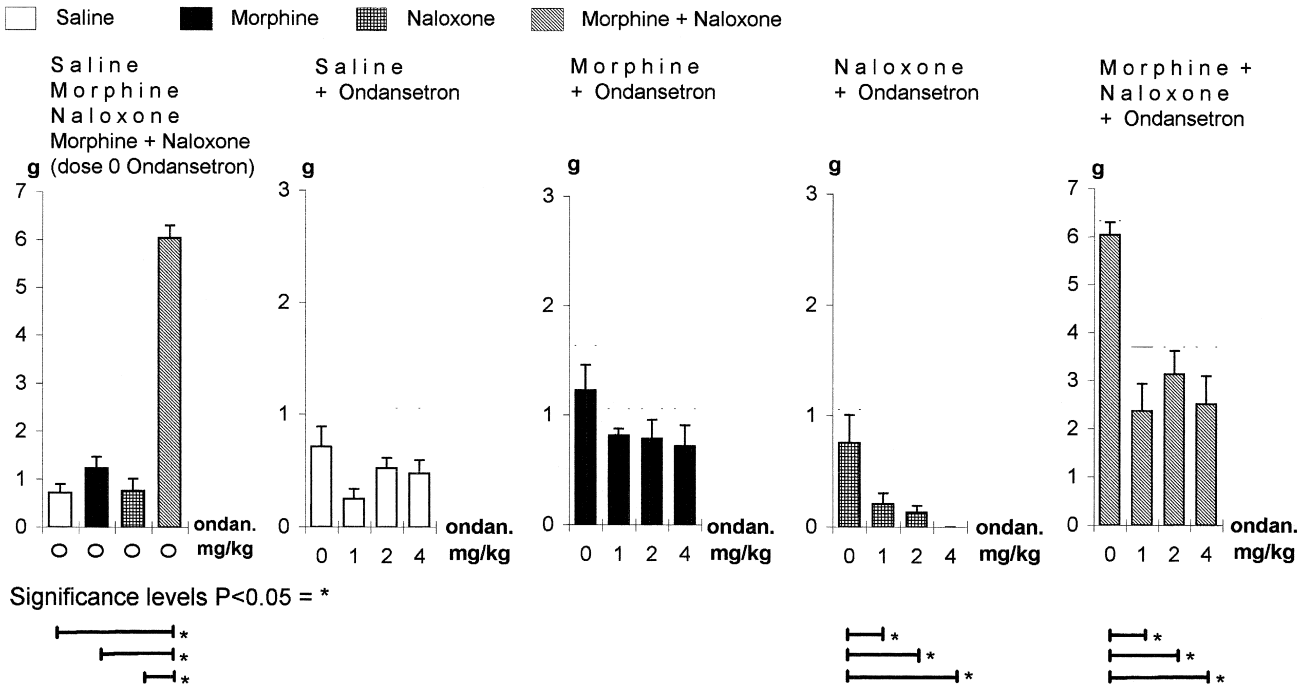

Fig. 2. Faeces excretion observed for 30 min in rats receiving saline, morphine, naloxone, morphine and naloxone and four doses of ondansetron given i.p. Each column represents the mean for 8 animals + S.E.

2.3. Statistical analysis

The values related to weights of faeces and urine or to rectal temperature and latency were subjected to parametric statistical analysis. Firstly, basal values (ondansetron dose 0) were compared among groups, using the Tukey test to validate the experimental conditions (Armitage, 1991). Secondly, a simple analysis was performed within each group receiving saline, morphine, naloxone or morphine plus naloxone by applying the Tukey test to the results with the four doses of ondansetron (0, 1, 2 and 4 mg/kg).

The signs, hypersalivation, jumping and wet-dog shakes, were evaluated only for groups receiving morphine plus naloxone and four doses of ondansetron (0, 1, 2 and 4 mg/kg) and were analysed with the Tukey test and the Armitage test (Armitage, 1991). Data related to faeces and urine excretion or to rectal temperature and latency times were analysed by means of an analysis of variance (ANOVA) according to the original $2 \times 2 \times 4$ factorial design (morphine, naloxone and four doses 0, 1, 2 and 4 mg/kg of ondansetron) (Armitage, 1991).

Statistical analysis was performed by using SAS software, version 6.08 (SAS Institute, SAS Campus Drive. Cary N.C. 27513 SAS Institute). The components were considered to be statistically significant at the level of 5%.

2.4. Drugs

Morphine was obtained from S.A.L.A.R.S. S.p.A. (Como). Naloxone was purchased from Sigma (Milan). Ondansetron was obtained from a commercial source. The drugs were dissolved in saline.

3. Results

3.1. Excretion of faeces

The changes in faeces excretion observed in all the groups of rats receiving saline, morphine, naloxone or morphine and naloxone are shown in Fig. 2. In the absence of ondansetron (dose 0) a marked increase of faeces excretion was observed in animals treated with morphine and naloxone when compared with all other groups ($P < 0.05$, Tukey test, Fig. 2). Ondansetron administration to animals receiving naloxone, or morphine combined with naloxone significantly decreased faeces excretion ($P < 0.05$, Tukey test).

Faeces excretion was altered highly significantly by the presence versus the absence of the individual factors: morphine ($P \leq 0.0001$), naloxone ($P \leq 0.0001$) or ondansetron ($P \leq 0.0001$).

The significant interaction of morphine × naloxone ($P \leq 0.0001$) demonstrated that morphine affected differently in the presence or absence of naloxone, the mean values of faeces excretion obtained from pooling the results from all ondansetron doses.

Furthermore, the presence or absence of morphine or naloxone significantly affected the faeces excretion values in the presence of different doses of ondansetron ($P \leq 0.0003$ for morphine × ondansetron interaction). Also the

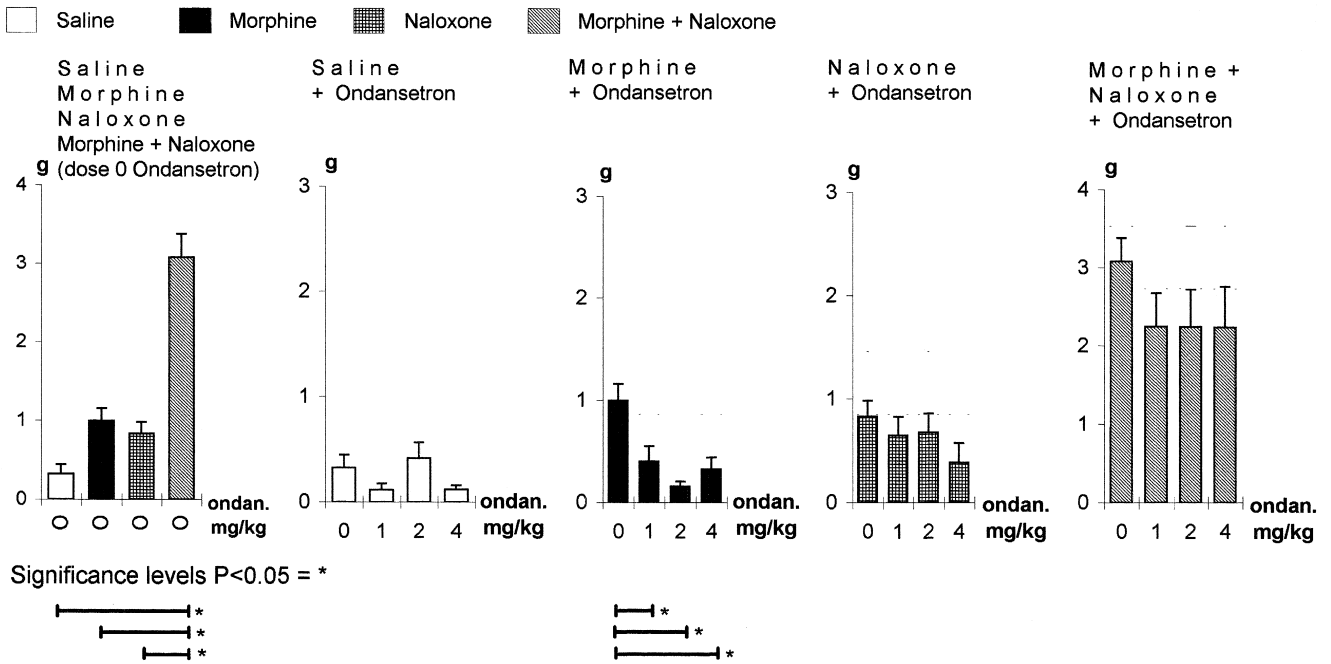

Fig. 3. Urine excretion observed for 30 min in rats receiving saline, morphine, naloxone, morphine and naloxone and four doses of ondansetron given i.p. Each column represents the mean for 8 animals + S.E.

effect of the presence or absence of naloxone on the dose–response profile of ondansetron was significant ($P \leq 0.0001$) for the naloxone × ondansetron interaction. The highly significant interaction of morphine × naloxone × ondansetron ($P \leq 0.0007$) demonstrated that the presence or absence of naloxone influenced the morphine effect on the ondansetron dose–response trend.

3.2. Excretion of urine

In the absence of ondansetron (dose 0) a marked increase in urine excretion in comparison with all other groups was observed in animals treated with morphine and naloxone combined ($P < 0.05$, Tukey test) (Fig. 3).

Ondansetron administration appeared to lower urine excretion only in the morphine group ($P < 0.05$, Tukey test).

The presence versus the absence of the individual factors, morphine ($P \leq 0.0001$), naloxone ($P \leq 0.0001$) and ondansetron ($P \leq 0.0125$) significantly affected the urine excretion values.

The interaction of morphine × naloxone was highly significant ($P \leq 0.0001$) and demonstrated that the modification of urine excretion levels, averaged over all the ondansetron dose levels, from absent to present naloxone, differed in a significant manner whether in the presence or absence of morphine.

The combined interactions between the different groups, with morphine × ondansetron, naloxone × ondansetron and morphine × naloxone × ondansetron were not significant.

3.3. Rectal temperature

In the absence of ondansetron (dose 0), rats treated with morphine exhibited increased rectal temperatures in comparison with other groups ($P < 0.05$, Tukey test). The animals receiving naloxone showed temperatures lower than other groups ($P < 0.05$, Tukey test) (Fig. 4). Rats receiving morphine and naloxone had rectal temperatures below the values observed in all the other groups ($P < 0.05$, Tukey test) (Fig. 4). Ondansetron administration to rats treated with morphine or naloxone decreased rectal temperature ($P < 0.05$, Tukey test).

The temperature levels were significantly affected by the various drugs: morphine ($P \leq 0.0014$), naloxone ($P \leq 0.0001$) and ondansetron ($P \leq 0.0019$). The interaction morphine × naloxone was highly significantly ($P \leq 0.0001$) related to rectal temperature and demonstrated that

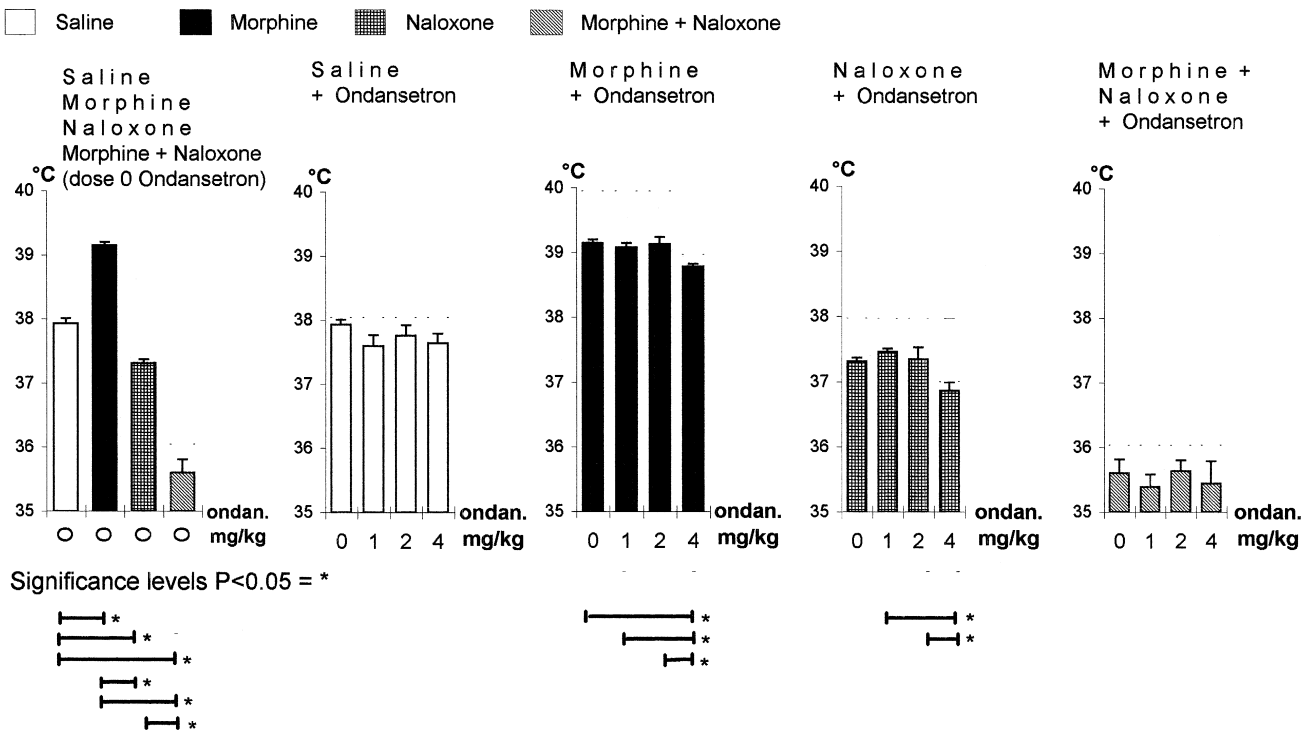

Fig. 4. Rectal temperature observed at the 30th min in rats receiving saline, morphine, naloxone, morphine and naloxone and four doses of ondansetron given i.p. Each column represents the mean for 8 animals + S.E.

morphine effects on temperature levels averaged over ondansetron doses differed in the presence and in the absence of naloxone.

The interactions morphine × ondansetron, naloxone × ondansetron, morphine × naloxone × ondansetron were not significant.

3.4. Tail-flick latencies

When the results for groups of animals receiving dose 0 of ondansetron were analysed, rats repeatedly treated with morphine exhibited latencies similar to those observed in the controls (Fig. 5). The group of animals receiving morphine and naloxone combined showed pain threshold values lower than those in all the other groups ($P < 0.05$, Tukey test). Ondansetron administration to rats treated with saline, morphine, naloxone, morphine and naloxone increased the tail-flick latency ($P < 0.05$, Tukey test).

The latencies were also significantly increased by the presence of morphine ($P \leq 0.0001$), or ondansetron ($P \leq 0.0001$) and decreased by naloxone ($P \leq 0.0001$). The presence or absence of morphine significantly affected the latency time values in the presence of different doses of ondansetron ($P \leq 0.0042$) for the morphine × ondansetron interaction. The effect of the presence or absence of naloxone on the dose–response profile of ondansetron was also significant ($P \leq 0.0406$) for the naloxone × ondansetron interaction.

3.5. Salivation

The animals repeatedly treated with morphine and subsequently with naloxone exhibited hypersalivation (2.63 ± 0.38 score) (Fig. 6). Ondansetron treatment did not significantly decrease the intensity of salivation.

3.6. Jumping

Jumping was observed in rats receiving morphine combined with naloxone (18.75 ± 5.91 jumps) (Fig. 6). Ondansetron significantly ($P < 0.05$, Tukey test) reduced the number of jumps in rats injected with morphine and naloxone (Fig. 6).

3.7. Wet-dog shakes

Wet-dog shakes were observed in animals receiving morphine and naloxone (5.5 ± 1.63 shakes) (Fig. 6). Ondansetron administration significantly ($P < 0.05$, Tukey test) affected the number of shakes in animals given morphine and naloxone (Fig. 6).

In addition, high percentages of the animals exhibiting hypersalivation (87.5%), jumping (100%) and wet-dog shakes (87.5%) were in the group receiving morphine combined with naloxone. The administration of ondansetron to rats receiving the morphine and naloxone

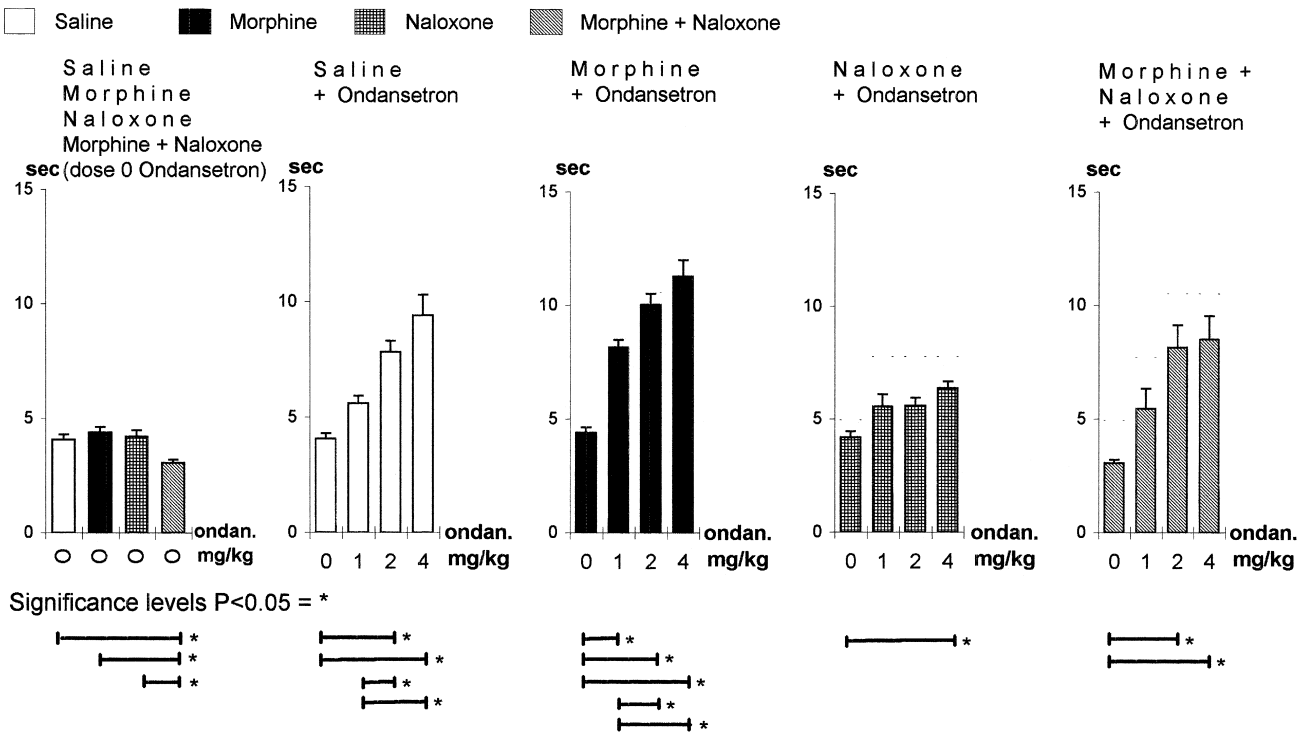

Fig. 5. Latency times observed at the 30th min in rats receiving saline, morphine, naloxone, morphine and naloxone and four doses of ondansetron given i.p. Each column represents the mean for 8 animals + S.E.

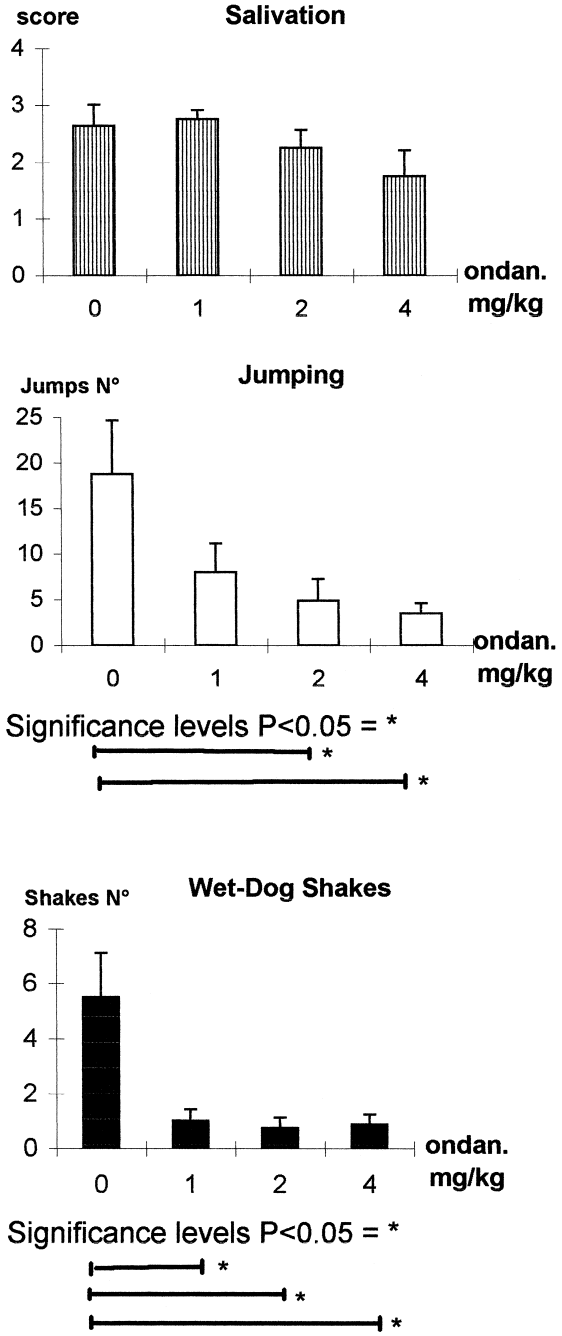

Fig. 6. Salivary secretion (score), jumping and wet-dog shakes (number of events) observed in animals receiving morphine and naloxone + ondansetron given i.p. at doses 0, 1, 2 and 4 mg/kg. Each column represents the mean for 8 animals + S.E.

combination significantly decreased the percentage of animals exhibiting jumping ($P < 0.02$), as evaluated by the Armitage test (χ^2 for slope).

4. Discussion

The combination of morphine and naloxone changed the rate of defecation and urination, the rectal temperature, latency times, salivation, jumping and wet-dog shakes as reported in literature (Martin and Jasinski, 1977; Redmond and Krystal, 1984; Bhargava, 1994). The data reported here not only confirm that naloxone precipitates physiological changes in morphine-dependent animals, but provides evidence that ondansetron administration prevents several of these withdrawal signs as discussed in detail below.

Ondansetron decreased faeces excretion. The antipropulsive effect exhibited by ondansetron in this experiment was in agreement with previous reports that this drug increases intestinal content transit time and lowers faeces excretion values (Talley et al., 1990; Lamers, 1991).

Ondansetron exerted an inhibitory effect on micturition: this activity was also attributable to a blockade of 5-HT_3 receptor stimulation of urine excretion (Corsi et al., 1991).

Ondansetron administration decreased the temperature in the morphine and in the naloxone groups. This effect can be explained by the observation that serotonin increases temperature in rodents (Carruba et al., 1979) through 5-HT_3 receptor activation (Mazzola-Pomietto et al., 1995) an effect which can be blocked by ondansetron as we now showed.

Ondansetron increased tail-flick latencies in all groups even though there was a slight effect of the presence or absence of morphine or naloxone, as is shown by significant morphine × ondansetron and naloxone × ondansetron interactions. Ondansetron appears to act through a mechanism which may involve 5-HT_3 receptor antagonist activity. This drug has been reported to exert analgesic activity by counteracting the nociceptive stimulation due to chemical agents (Giordano and Dyche, 1989), in particular to peripherally administered serotonin (Sufka et al., 1992), through 5-HT_3 receptor blockade, which is associated with nociceptive transmission.

In rats exhibiting hypersalivation due to morphine and naloxone treatment (Kromer, 1993; Redmond and Krystal, 1984), ondansetron administration did not affect the increase, presumably because 5-HT_3 receptors are not involved in salivation control.

Ondansetron administration significantly decreased the number of jumps in rats given morphine and naloxone. Since jumping activity is attributable to supersensitivity of dopamine receptors caused by morphine (Lal, 1975), the ondansetron effect was probably due to blockade of 5-HT_3 receptors, affecting the activation of dopaminergic neurones associated with locomotor and jumping behaviour (Acquas et al., 1988; Higgins et al., 1992; Pei et al., 1993; Borg and Taylor, 1994).

Ondansetron administration to animals receiving combined morphine and naloxone lessened the shaking behaviour, presumably because 5-HT_3 receptors were causally involved in wet-dog shakes, since this behaviour is attributable either to serotoninergic mechanisms (Gulati and Bhargava, 1989, 1990; Kruszewska and Langwinski, 1983) or to other transmitter involvement (Cowan, 1993).

5. Conclusions

In the present study, ondansetron administration affected several individually analysed morphine withdrawal signs such as alteration of faeces excretion, pain threshold levels and also behaviour such as jumping and wet-dog shakes, although other authors have reported it to have no influence on salivation and wet-dog shakes in rats (Higgins et al., 1991). The effective doses of ondansetron in the present study were similar to those used in human therapy. Other reports have shown that ondansetron decreases self-administration of morphine in rats (Hui et al., 1993). A report appeared on the suppressive activity exerted by ondansetron on some abstinence behavioural symptoms grouped together in rats, such as wet-dog shakes, diarrhoea, ptosis, chattering teeth, chewing, paw tremor and irritability to touch (Hui et al., 1996). Furthermore, 5-HT_3 receptor antagonists have been proposed for the control or suppression of the behavioural symptoms associated with withdrawal from drugs of abuse such as nicotine and cocaine, (Costall and Naylor, 1992). Although ondansetron has been administered to addicts in an attempt to reduce opiate craving, this was unfortunately without success (Sell et al., 1995). Ondansetron has also been utilised with guanfacine for the control of gastrointestinal hypermotility observed in heroin addicts in the ultrarapid opioid detoxification procedure (UROD) (Legarda and Gossop, 1994).

For all the above-mentioned reasons, ondansetron may be a candidate for effective control of several acute withdrawal symptoms in heroin addicts.

Acknowledgements

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