ELSEVIER

European Journal of Pharmacology 287 (1995) 263–269

ejp

Effects of granisetron, a 5-HT_3 receptor antagonist, on morphine-induced potentiation of brain stimulation reward

Pierre-Paul Rompré [a,*], Rita Injoyan [a], Jim J. Hagan [b]

[a] *Centre de Biomédecine, Hôpital du Sacré-Coeur et Département de psychiatrie, Université de Montréal, Montréal (Québec), Canada*
[b] *SmithKline Beecham Pharmaceuticals, New Frontiers Science Park, Third Avenue, Harlow, Essex CM19 5AW, UK*

Received 27 April 1995; revised 1 August 1995; accepted 8 August 1995

Abstract

Using the curve-shift method, we studied the effects of four doses (0.003, 0.03, 0.3 and 3 mg/kg, s.c.) of granisetron (endo-*N*-(9-methyl-9-azabicyclo[3.3.1]non-3-yl)-1-methyl-1*H*-indazole-3-carboxamide hydrochloride), a selective 5-hydroxytryptamine$_3$ (5-HT_3) receptor antagonist, on the potentiation of brain stimulation reward by microinjection of 2.5 μg/0.25 μl of morphine sulphate (7,8-didehydro-4,5-epoxy-17-methylmorphinan-3,6-diol sulphate) into the ventral tegmental area. As previously reported, morphine produced a significant reduction in reward threshold without altering maximal rates of responding. Granisetron attenuated the potentiating effect of morphine at the highest dose and failed to alter reward threshold or maximal rates of responding when given alone, except at the lowest dose where a small and statistically significant increase in threshold was found. These results provide additional evidence that 5-HT_3 receptor antagonists may reduce the rewarding effect of opiates and do not impair the ability to produce operant responses. The weak attenuation observed with granisetron alone suggests that 5-HT_3 receptors are unlikely to constitute an important influence on the directly stimulated reward-relevant pathway(s).

Keywords: 5-HT_3 receptor; Dopamine; Granisetron; Morphine; Reward

1. Introduction

Previous studies have shown that opioid receptors in the ventral tegmental area mediate, at least in part, the rewarding effect of morphine and heroin. Microinjections of morphine or opioid receptor agonists into the ventral tegmental area, for instance, sustain self-administration (Bozarth and Wise, 1981a; Devine and Wise, 1994), produce a conditioned place preference (Phillips and LePiane, 1980) and potentiate brain stimulation reward (Rompré and Wise, 1989a). In addition, Britt and Wise (1983) showed that blockade of opioid receptors in the ventral mesencephalon leads to compensatory increases in heroin self-administration which is interpreted as a decrease in reward.

Several lines of evidence suggest that the rewarding effect of opioid receptor activation in the ventral tegmental area is mediated by an increase in dopamine neurotransmission. First, systemic or local injection of morphine into the ventral tegmental area stimulates dopamine cell firing (Gysling and Wang, 1983) and increases extracellular levels of dopamine in the nucleus accumbens (Imperato and Angelucci, 1989; Leone et al., 1991), a brain region involved in brain stimulation and drug mediated reinforcement (see Wise and Rompré, 1989). Second, place preference induced by morphine or heroin is attenuated by dopamine receptor blockers and by neurotoxic lesions of dopamine neurons (Bozarth and Wise, 1981b; Phillips et al., 1982, 1983). Third, microinjection of morphine into the ventral tegmental area reverses, up to a point, the attenuating effect of pimozide on brain stimulation reward, suggesting that the increase in dopamine impulse flow produced by morphine counteracts the blockade of dopamine receptors (Rompré and Wise, 1989a).

It has been shown that the action of opioids on dopamine neurotransmission is indirect and results from the inhibition of a tonic γ-aminobutyric acid (GABA) input to dopamine neurons. Unlike dopamine

* Corresponding author. Centre de Biomédecine, Hôpital du Sacré-Coeur, 5400 Boulevard Gouin Ouest, Montréal (Québec), Canada, H4J 1C5. Fax: (514) 338-2694.

SSDI 0014-2999(95)00497-1

neurons, non-dopamine neurons are hyperpolarized by opioids (Johnson and North, 1992a), and infusion of morphine through a microdialysis probe implanted into the ventral tegmental area produces a significant reduction of extracellular levels of GABA and increases dopamine (Klitenick et al., 1992). Recent behavioral and neurochemical studies suggest that 5-HT_3 receptors may also play a role in the stimulation of dopamine impulse flow produced by opioids. Systemic administration of selective 5-HT_3 receptor antagonists, such as ICS 205-930 (tropisetron), BRL 43694 (granisetron) and GR 38032F (ondansetron), reduces the increase in locomotion, and the increase in dopamine release and metabolism, produced by systemic morphine (Imperato and Angelucci, 1989; Pei et al., 1993). Furthermore, tropisetron attenuates morphine-induced conditioned place preference (Carboni et al., 1988) while ondansetron reduces morphine self-administration (Hui et al., 1993). Although the exact site of action of the 5-HT_3 receptor antagonists is still unknown, some results suggest that they may act in the ventral tegmental area. Ventral tegmental neurons receive synaptic inputs from 5-HT terminals (Hervé et al., 1987), and Perry (1990) showed evidence of 5-HT_3 binding sites in this region. In addition, Imperato and Angelucci (1989), using an in vivo microdialysis technique, reported that microinjections of tropisetron into the ventral tegmental area reverse morphine-induced dopamine release in the nucleus accumbens while perfusion through the microdialysis probe did not alter the morphine effect. These results suggest that 5-HT_3 receptor antagonists may attenuate the stimulant effect of opioids on dopamine cell firing. Hence, it has been reported that pretreatment with ondansetron prevents morphine-induced stimulation of dopamine cell firing (Christoffersen et al., 1988) a finding, however, that was not replicated with BRL 46470A (an analog of granisetron) and tropisetron (Gifford and Wang, 1994).

In this study, we investigated the role of 5-HT_3 receptors in opioid reward by testing the effects of several doses of granisetron on the potentiation of brain stimulation reward produced by microinjection of morphine into the ventral tegmental area. Brain stimulation reward is highly sensitive to changes in dopamine neurotransmission and, just like opiate reward, is thought to involve those dopamine neurons that mainly project to the nucleus accumbens (see Wise and Rompré, 1989).

2. Materials and methods

2.1. Subjects

The subjects were adult male hooded rats weighing 300–350 g at the time of surgery. They were housed individually with free access to food and water, and were maintained on 14 h/10 h light-dark cycle.

2.2. Surgery

A moveable stimulating electrode (Kinetrods, SME-01) and a guide injection cannula (0.7 mm, o.d.) were unilaterally implanted (under sodium pentobarbital anesthesia, Somnotol, 65 mg/kg, i.p.) in the caudal mesencephalic central gray and above the ventral tegmental area. With the incisor bar set to hold the skull horizontal, the stereotaxic coordinates were: 7.6 mm posterior to bregma, 0.0 lateral and 6.5 mm below the skull for the electrode, and 5.2 mm posterior, 2.4 lateral and 6.8 ventral for the guide cannula; the cannula was angled at 15° off the vertical to avoid reflux of injected fluid into the mesencephalic central gray. An indifferent electrode was wrapped around four skull screws that were threaded into the skull and the entire assembly was fixed with acrylic dental cement (Rompré and Wise, 1989a).

2.3. Training procedure and behavioral testing

After at least 1 week of recovery following surgery, animals were trained to self-stimulate using procedures previously described (Rompré and Gratton, 1993). With the current intensity held constant (train of cathodal, rectangular pulses of 0.1 ms in duration), the animals were then trained to bar press during several discrete 45 s trials, each separated by a 25 s interval during which the bar was disconnected. The beginning of each trial was signaled by five trains of non-contingent priming stimulation delivered once per second. For each animal, the stimulation frequency on the first trial was set at 115 Hz and was lowered on each subsequent trial by approximately 15% until it reached 20 Hz. Estimates of reward threshold were derived from the resulting function relating the rate of bar pressing to the stimulation frequency (rate-frequency function). Drug and vehicle tests were performed on separate daily test sessions, each of which consisted of two test periods. During the first test period, three baseline rate-frequency functions were determined: the first function was considered as a warm-up period and was therefore not included in the analysis. Immediately following the baseline test period, each animal was centrally injected with vehicle or 2.5 μg/0.25 μl of morphine sulphate, followed 30 min later by a systemic vehicle or granisetron injection; the systemic injection was performed 30 min after the central injection based on a previous study showing that the potentiation of brain stimulation reward by morphine occurred only 30–45 min after the injection (Rompré and Wise, 1989a). The cannula used for the microinjection (0.3 mm, o.d.) extended 1 mm beyond the tip of the guide cannula

and was connected with polyethylene tubing to a 1 μl microsyringe. A 0.25 μl volume of morphine or vehicle was injected with a microinfusion pump over a period of 60 s. Beginning 30 min after the microinjection, four rate-frequency functions were determined over the second test period that lasted 70 min. Four groups of animals were used. One group was assigned to each dose of granisetron and animals in each group were tested on four occasions following: central vehicle + systemic vehicle; central morphine + systemic vehicle; central vehicle + systemic granisetron and central morphine + systemic granisetron. The four doses of granisetron were: 0.003 (group 1), 0.03 (group 2), 0.3 (group 3) and 3.0 mg/kg, s.c. (group 4). The order of treatments was randomized in each group and at least 7 days separated each drug test.

2.4. Drugs

Granisetron (endo-1-methyl-*N*-(9-methyl-9-aza-bicyclo[3.3.1]non-3-yl)-1*H*-indazole-3-carboxamide hydrochloride) and morphine sulphate (7,8-didehydro-4,5-epoxy-17-methylmorphinan-3,6-diol sulphate) were dissolved in water and in Ringer's solution respectively. Morphine sulphate solutions (10 μg/μl) were stored frozen at −20°C in 50 μl aliquots and thawed just before testing. Doses are expressed as salt.

2.5. Histological analysis

At the completion of the study, animals were deeply anesthetized with sodium pentobarbital and the stimulation site was marked by passing 100 μA anodal direct current for 15 s; they were then intracardially perfused with saline and 10% formalin solution. The brains were removed, soaked for approximately 12 h in a 10% formalin solution containing ferro-and ferricyanide (3%), and then stored for several days in 10% formalin. They were subsequently sliced in serial 40 μm sections which were stained with a formal-thionin solution. Location of stimulation and injection sites were determined under light microscopic examination; only animals with injection sites within the ventral tegmental area, between 4.8 and 5.8 mm posterior to bregma, were included in the analysis.

2.6. Data analysis

A reward threshold was estimated from each rate-frequency function and was operationally defined as the frequency required to sustain half-maximal rate of bar presses. Thresholds and maximal rates were expressed as the percentage of pre-injection value and statistical significance was determined with a one-way analysis of variance with repeated measures. Post-hoc comparisons among means were performed with Newman-Keuls tests.

3. Results

The time-dependent (top panel) and average (bottom panel) changes in reward threshold obtained following each drug treatment, and from each group of animals tested, are shown in the figures. In each group, morphine produced a 15–20% decrease in threshold. Granisetron failed to attenuate the facilitation effect of morphine at doses of 0.003–0.3 mg/kg (Fig. 1, Fig. 2, Fig. 3) but blocked this facilitation at 3 mg/kg (Fig. 4). The analysis of variance performed on the average data (bottom panels) revealed, for each group, a significant effect of treatments (0.003 mg/kg, $F(3,12) = 26.0$, $P < 0.001$; 0.03 mg/kg, $F(3,15) = 7.2$, $P < 0.004$; 0.3 mg/kg, $F(3,15) = 5.5$, $P < 0.01$; 3 mg/kg, $F(3,12) = 7.8$, $P < 0.004$). Post-hoc tests showed that the decrease in threshold produced by morphine alone was statistically

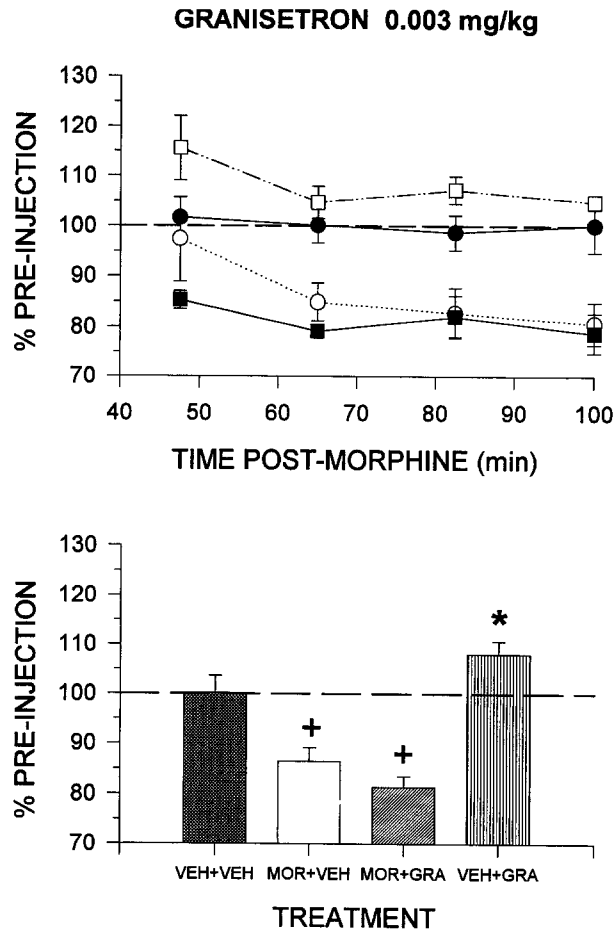

Fig. 1. Time course (top panel) and average (bottom panel) changes in reward threshold following vehicle + vehicle (filled circle), morphine + vehicle (open circle), morphine + granisetron (filled square) and vehicle + granisetron (open square) treatments. Results are expressed as percentage of pre-injection threshold and represent the means ± S.E.M ($n = 5$). Each point on the x-axis (top panel) corresponds to the end of each *R-F* function determination. The $^{+}$ and * denote statistical significance ($P < 0.01$ and $P < 0.05$, respectively) compared to vehicle + vehicle treatment. Abbreviations: GRA, granisetron; MOR, morphine; VEH, vehicle.

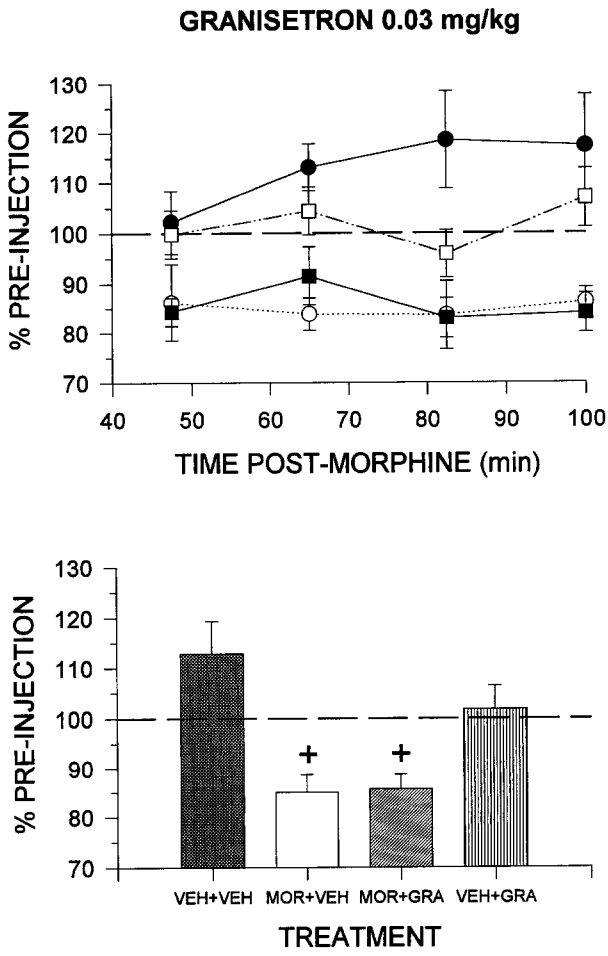

Fig. 2. Time course (top panel) and average (bottom panel) changes in reward threshold following each drug treatments (refer to Fig. 1 for details). Results represent the means ± S.E.M ($n = 6$). The $^{+}$ denotes statistical significance ($P < 0.01$) compared to vehicle + vehicle treatment.

significant when compared to threshold after vehicle treatments in every group ($P < 0.05$). In the group treated with 3 mg/kg of granisetron, average threshold after morphine + granisetron was not statistically different from threshold after vehicle treatments but was different than morphine + vehicle (Fig. 4, bottom panel). Granisetron alone failed to alter threshold at doses of 0.03–3 mg/kg, but produced a small (8%) and statistically significant increase compared to vehicle treatments at 0.003 mg/kg ($P < 0.05$).

The analysis of variance performed on the average rates of maximal responding revealed that this measure was not altered by morphine, by granisetron alone, nor by a combination of these treatments (data not shown).

4. Discussion

The present results replicate previous findings showing that microinjection of morphine into the ventral tegmental area produces a significant reduction in brain stimulation reward threshold as measured with the curve-shift method (Bauco et al., 1993; Rompré and Wise, 1989a). Several lines of evidence support the hypothesis that the potentiation of brain stimulation reward by morphine is due to an increase in dopamine impulse flow. First, activation of opioid receptors in the ventral tegmental area stimulates dopamine cell firing (Gysling and Wang, 1983; Johnson and North, 1992a) and produced significant increases in extracellular levels of dopamine in the nucleus accumbens (Leone et al., 1991). Second, brain stimulation reward is highly sensitive to changes in dopamine impulse flow; it is inhibited by dopamine receptor blockers and enhanced by indirect dopamine agonists such as amphetamine and GBR 12909 (Gallistel and Karras, 1984; Rompré and Gratton, 1993). Brain stimulation reward is also potentiated by microinjections of neurotensin into the ventral tegmental area, a treatment that, just like morphine, stimulates dopamine cell firing and release (see Rompré and Gratton, 1993), and it is inhibited by

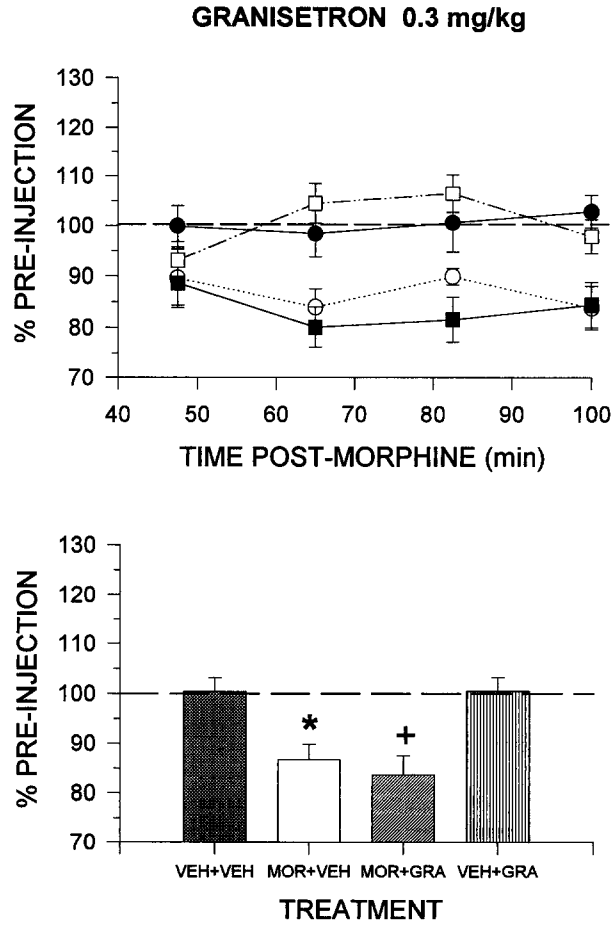

Fig. 3. Time course (top panel) and average (bottom panel) changes in reward threshold following each drug treatments (refer to Fig. 1 for details). Results represent the means ± S.E.M ($n = 6$). The $^{+}$ and * denote statistical significance ($P < 0.01$ and $P < 0.05$, respectively) compared to vehicle + vehicle treatment.

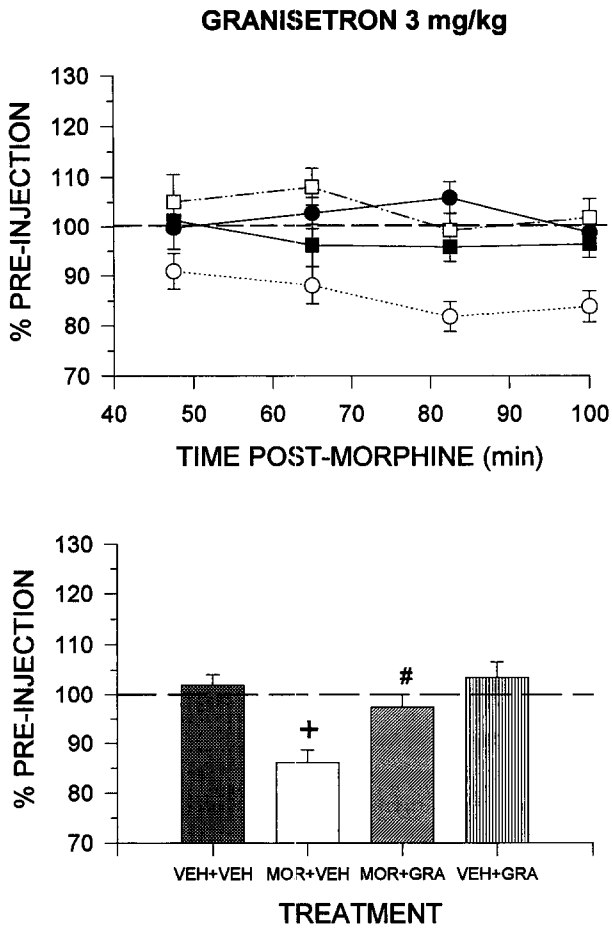

Fig. 4. Time course (top panel) and average (bottom panel) changes in reward threshold following each drug treatments (refer to Fig. 1 for details). Results represent the means ± S.E.M ($n = 5$). The $^{+}$ denotes statistical significance compared to vehicle + vehicle treatment ($P < 0.01$); the $^{\#}$ denotes statistical significance between morphine + vehicle and morphine + granisetron ($P < 0.05$).

microinjections of muscimol into the ventral tegmental area (Rompré and Wise, 1989b), a GABA receptor agonist known to hyperpolarize dopamine neurons (Johnson and North, 1992b). Third, rewarding stimulations increase dopamine release in the nucleus accumbens (Miliaressis et al., 1991) and microinjections of dopamine receptor blockers and amphetamine directly into the nucleus accumbens attenuate and potentiate, respectively, brain stimulation reward (Kurumiya and Nakajima, 1988; Ranaldi and Beninger, 1994). Taken together, these results provide strong evidence that mesoaccumbens dopamine neurons constitute important elements of the reward relevant pathways.

The main objective of this study was to test whether systemic injection of the selective 5-HT_3 receptor antagonist, granisetron, attenuates the reward potentiating effect of morphine injected into the ventral tegmental area. Previous studies have shown that selective 5-HT_3 receptor antagonists attenuate morphine-induced dopamine release in the nucleus accumbens (Imperato and Angelucci, 1989; Pei et al., 1993) and morphine self-administration (Hui et al., 1993), suggesting that blockade of 5-HT_3 receptors may attenuate the rewarding effect of opioids. The present results are consistent with this hypothesis as granisetron blocked the reward potentiating effect of morphine. The effective dose of granisetron in this study, however, is larger than the effective dose of other 5-HT_3 receptor antagonists reported in some previous neurochemical studies. Imperato and Angelucci (1989) and Carboni et al. (1988, 1989) showed that tropisetron was effective at blocking morphine-induced dopamine release and morphine-induced place preference at doses as low as 0.03 and 0.1 mg/kg. Pei et al. (1993), however, did not replicate these findings and reported that a dose of at least 1 mg/kg (granisetron, tropisetron and ondansetron) was required to attenuate the behavioral and neurochemical effects of morphine. The reason for this discrepancy is not clear, but as pointed out by the authors, methodological differences may account for such a difference in sensitivity. The low potency of granisetron in the present study is unlikely to be due to the fact that morphine was administered before granisetron as our results are consistent with those reported by Pei et al. (1993) who injected granisetron prior to morphine. At doses lower than 1.0 mg/kg, they did not find any effect of granisetron on morphine-induced dopamine release and we did not find any attenuation on the reward potentiating effect of morphine. Our results are also consistent with previous electrophysiological findings showing that at 1.0 mg/kg ondansetron attenuates the stimulant effect of morphine on dopamine cell firing. (Christoffersen et al., 1988). As pointed out by Pei et al. (1993), it is likely that even at this high dose, granisetron should still show specificity for the 5-HT_3 receptor as it has a high affinity for this receptor and a very low affinity for other receptors (Kilpatrick et al., 1987; Sanger and Nelson, 1989). The similar potentiation effect of morphine observed across the four groups of animals tested in this study excludes the possibility that the effectiveness of granisetron at the high dose is due to differences in sites of injection, and that is consistent with the histological results.

Although granisetron blocked the reward potentiating effect of morphine at 3 mg/kg, it had no effect by itself and failed to alter threshold at doses in excess of 0.03 mg/kg, findings that are in agreement with previous reports (Hatcher et al., 1995; Greenshaw, 1993). At the lowest dose tested, however, we found a small and statistically significant increase in threshold. This effect was lost at higher doses, a phenomenon previously reported in various behavioral paradigms for other 5-HT_3 receptor antagonists (Jones et al., 1988; Domeney et al., 1991; Tomkins et al., 1995); it would be interesting to determine whether lower doses of

granisetron would produce higher increases in threshold. Such an increase in threshold at the low dose is hard to reconcile with its lack of effect on the reward potentiating effect of morphine. Since brain stimulation reward itself has been shown to stimulate dopamine release, this suggests that the mechanism by which rewarding stimulations increase dopamine release is different from that involved in morphine-induced dopamine release. The weak attenuation observed at the low dose with granisetron alone suggests that 5-HT_3 receptors are unlikely to constitute an important influence on the directly stimulated reward-relevant pathway(s).

Previous studies suggest that 5-HT_3 receptor antagonists are not effective, or are less potent, at attenuating dopamine release stimulated by drugs that act through an impulse-independent mechanism, such as amphetamine and cocaine, but are more effective against drugs that act through an impulse-dependent mechanism such as morphine, nicotine and ethanol (see Hagan et al., 1993; Greenshaw, 1993). This suggests that 5-HT_3 receptor antagonists may act through the circuitry involved in modulating dopamine impulse flow. Since morphine-induced dopamine release is blocked by microinjections of tropisetron into the ventral tegmental area, we hypothesized that 5-HT_3 receptor antagonists may act locally in this region. This hypothesis was further supported by studies showing that 5-HT terminals established synaptic inputs with ventral tegmental neurons (Hervé et al., 1987) and that 5-HT_3 binding sites are present in this region (Perry, 1990). As morphine was injected directly into the ventral tegmental area, the potentiation of brain stimulation reward could only be accounted for by the action of morphine in this region. It is known that morphine increases dopamine cell firing by inhibiting a tonic GABAergic input to dopamine neuron, and one possible explanation for the attenuating effect of granisetron is that blockade of 5-HT_3 receptors results in an increase in GABA release that counteracts the morphine effect, an hypothesis originally suggested by Pei et al. (1993).

Another explanation is that blockade of 5-HT_3 receptors potentiates the short-and/or long-loop negative feedback inhibitory inputs to dopamine neurons. It is well known that the firing rate of dopamine neurons is modulated by autoreceptors (short loop) and by an inhibitory feedback pathway from dopamine terminal regions (long loop) that are both under the control of dopamine (see Chiodo, 1988). Direct or indirect dopamine agonists inhibit while dopamine receptor antagonists stimulate dopamine cell firing by acting through these feedback mechanisms. Hence, 5-HT_3 receptor antagonists potentiate apomorphine-induced inhibition of dopamine cell firing (Ashby et al., 1994; Uzzle and Haskins, 1991) and attenuate haloperidol-induced dopamine release (Carboni et al., 1989). Such a potentiation of the inhibitory inputs to dopamine neurons could explain why 5-HT_3 receptor antagonists are more effective at blocking impulse-dependent dopamine release. Furthermore, because the effect of 5-HT_3 receptor antagonists on basal dopamine release and firing and on spontaneous dopamine-dependent behaviors is, at best, weak, it would also suggest that 5-HT_3 receptors indirectly modulate the negative feedback inputs to dopamine neurons.

The lack of effects of granisetron on maximal rates of responding suggests that 5-HT_3 receptor blockade does not alter gross aspects of performance in an operant responding paradigm and is devoid of behavioral side-effects. This implies that changes in operant responding for reward produced by 5-HT_3 receptor antagonists are unlikely due to a decrease in the ability of the animals to perform the task and reliably reflect changes in the rewarding value of the stimulus.

Acknowledgements

The authors would like to thank Mrs Melina Serio for her excellent technical assistance. This work was supported by a grant from the Medical Reseach Council (MRC) of Canada. RI was supported by a fellowship from MRC and PPR is a Chercheur-boursier from le Fonds de la recherche en santé du Québec. Granisetron was kindly provided by SmithKline Beecham Pharmaceuticals (Harlow, UK).

References

Ashby, C.R., Jr., Y. Minabe, A. Toor, L.D. Fiskin, M.I. Granoff and R.Y. Wang, 1994, Effect produced by acute and chronic administration of the selective 5-HT_3 receptor antagonist BRL46470 on the number of spontaneously active midbrain dopamine cells in the rat, Drug, Dev. Res. 31, 228.

Bauco, P., Y. Wang and R.A. Wise, 1993, Lack of sensitization or tolerance to the facilitating effect of ventral tegmental area morphine on lateral hypothalamic brain stimulation reward, Brain Res. 617, 303.

Bozarth, M. and R.A. Wise, 1981a, Intracranial self-administration of morphine into the ventral tegmental area in rats, Life Sci. 28, 551.

Bozarth, M. and R.A. Wise, 1981b, Heroin reward is dependent on a dopaminergic substrate, Life Sci. 29, 1881.

Britt, M.D. and R.A. Wise, 1983, Ventral tegmental site of opiate reward: antagonism by a hydrophyllic opiate receptor blocker, Brain Res. 258, 105.

Carboni, E., E. Acquas, P. Leone, L. Perrazzani and G. Di Chiara, 1988, 5-HT_3 receptor antagonists block morphine-and nicotine-induced place-preference conditioning, Eur. J. Pharmacol. 151, 159.

Carboni, E., E. Acquas, R. Frau and G. Di Chiara, 1989, Differential inhibitory effects of a 5-HT_3 antagonist on drug-induced stimulation of dopamine release, Eur. J. Pharmacol. 164, 515.

Christoffersen, C.L., K.A. Serpa and L.T. Meltzer, 1988, Effects of the serotonin-3 (5HT-3) antagonist, GR38032F, on midbrain dopamine (DA) neurons, Soc. Neurosci. Abstr. 14, 847.

Chiodo, L.A., 1988, Dopamine-containing neurons in the mammalian central nervous system: electrophysiology and pharmacology, Neurosci Biobehav. Rev. 12, 49.

Devine, D.P. and R.A. Wise, 1994, Self-administration of morphine, DAMGO and DPDPE into the ventral tegmental area of rats, J. Neurosci. 14, 1978.

Domeney, A.M., B. Costall, P.A. Gerrard, D.N.C. Jones, R.J. Naylor and M.B. Tyers, 1991, The effect of ondansetron on cognitive performance in the marmoset, Pharmacol. Biochem. Behav. 38, 169.

Gallistel, C.R. and D. Karras, 1984, Pimozide and amphetamine have opposing effects on the reward summation function, Pharmacol. Biochem. Behav. 20, 73.

Gifford, A.N. and R.Y. Wang, 1994, The effect of 5-HT_3 receptor antagonists on the morphine-induced excitation of A10 dopamine cells: electrophysiological studies, Brain Res. 638, 325.

Greenshaw, A.J., 1993, Behavioral pharmacology of 5-HT_3 receptor antagonists: a critical update on therapeutic potential, Trends Pharmacol. Sci. 14, 265.

Gysling, K. and R.Y. Wang, 1983, Morphine-induced activation of A10 dopamine neurons in the rat, Brain Res. 277, 119.

Hagan, R.M., G.J. Kilpatrick and M.B. Tyers, 1993, Interactions between 5-HT_3 receptors and cerebral dopamine function: implications for the treatment of schizophrenia and psychoactive substance abuse, Psychopharmacology 112, S68.

Hatcher, J.P., P. Boyland and J.J. Hagan, 1995, The 5-HT_3 receptor antagonists, granisetron and ondansetron, do not affect cocaine induced shifts in intracranial self-stimulation thresholds, Psychopharmacology (in press).

Hervé, D., V.M. Pickel, T.H. Joh and A. Beaudet, 1987, Serotonin axon terminals in the ventral tegmental area of the rat: fine structure and synaptic input to dopaminergic neurons, Brain Res. 435, 71.

Hui, S.-C. G., E.L. Sevilla, and C.W. Ogle, 1993, 5-HT_3 antagonists reduce morphine self-administration in rats, Br. J. Phamacol. 110, 1341.

Imperato, A. and L. Angelucci, 1989, 5-HT_3 receptors control dopamine release in the nucleus accumbens of freely moving rats, Neurosci. Lett. 101, 214.

Johnson, S.W. and A.R. North, 1992a, Opioids excite dopamine neurons by hyperpolarization of local interneurons, J. Neurosci. 12, 483.

Johnson, S.W. and A.R. North, 1992b, Two types of neurone in the rat ventral tegmental area and their synaptic inputs, J. Physiol. 450, 455.

Jones, B.J., B. Costall, A.M. Domeney, M.E. Kelley, R.J. Naylor, N.R. Oakley and M.B. Tyers, 1988, The potential anxiolytic activity of GR38032F, a 5-HT_3-receptor antagonist, Br. J. Pharmacol. 93, 985.

Kilpatrick, G.J., B.J. Jones and M.B. Tyers, 1987, Identification and distribution of 5-HT_3 receptors in rat using radioligand binding, Nature 330, 746.

Klitenick, M.A., P. De Witte and P.W. Kalivas, 1992, Regulation of somatodendritic dopamine release in the ventral tegmental area by opioids and GABA: an in vivo microdialysis study, J. Neurosci. 12, 2623.

Kurumiya, S. and S. Nakajima, 1988, Dopamine D1 receptors in the nucleus accumbens: involvement in the reinforcing effect of tegmental stimulation, Brain Res. 448, 1.

Leone, P., D. Pocock, and R.A. Wise, 1991, Morphine-dopamine interaction: Ventral tegmental morphine increases nucleus accumbens dopamine release, Pharmacol. Biochem. Behav. 39, 469.

Miliaressis, E., C. Emond and Z. Merali, 1991, Re-evaluation of the role of dopamine in intracranial self-stimulation using in vivo microdialysis, Behav. Brain Res. 46, 43.

Pei, Q., T. Zetterström, R.A. Leslie and D.G. Grahame-Smith, 1993, 5-HT_3 receptor antagonists inhibit morphine-induced stimulation of mesolimbic dopamine release and function in the rat, Eur. J. Pharmacol. 230, 63.

Perry, D.C., 1990, Autoradiography of [^{3}H]quipazine in rodent brain, Eur. J. Pharmacol. 187, 75.

Phillips, A.G. and F.G. LePiane, 1980, Reinforcing effects of morphine microinjection into the ventral tegmental area, Pharmacol. Biochem. Behav. 12, 965.

Phillips, A.G., C. Spyraki and H.C. Fibiger, 1982, Conditioned place preference with amphetamine and opiates as reward stimuli: attenuation by haloperidol, in: The Neural Basis of Feeding and Reward, eds. B.G. Hoebel and D. Novin (Haer Institute, Brunswick) p. 455.

Phillips, A.G., F.G. LePiane and H.C. Fibiger, 1983, Dopaminergic mediation of reward produced by direct injection of enkephalin into the ventral tegmental area of the rat, Life Sci. 33, 2505.

Ranaldi, R. and R.J. Beninger, 1994, Rostral-caudal differences in effects of nucleus accumbens amphetamine on VTA ICSS, Brain Res. 642, 251.

Rompré, P.-P. and A. Gratton, 1993, Mesencephalic microinjections of neurotensin-(1–13) and its C-terminal fragment, neurotensin-(8–13), potentiate brain stimulation reward, Brain Res. 616, 154.

Rompré, P.-P. and R.A. Wise, 1989a, Opioid-neuroleptic interaction in brainstem self-stimulation, Brain Res. 477, 144.

Rompré, P.-P. and R.A. Wise, 1989b, Behavioral evidence for midbrain dopamine depolarization inactivation, Brain Res. 477, 152.

Sanger, G.J. and D.R. Nelson, 1989, Selective and functional 5-hydroxytryptamine$_3$ receptor antagonism by BRL 43694 (granisetron), Eur. J. Pharmacol. 159, 113.

Tomkins, D.M., A.D. Le and E.M. Sellers, 1995, Effects of the 5-HT3 antagonist ondansetron on voluntary ethanol intake in rats and mice maintained on a limited access procedure, Psychopharmacology 117, 479.

Uzzle, C.W. and J.T. Haskins, 1991, The effect of WAY-100,289, a novel 5-HT3 receptor antagonist, on A9 and A10 dopamine (DA) neuronal activity: acute and chronic studies, Soc. Neurosci. Abstr. 17, 599.

Wise, R.A. and P.-P. Rompré, 1989, Brain dopamine and reward, Ann. Rev. Psychol. 40, 191.