ELSEVIER

European Journal of Pharmacology 303 (1996) 1–12

ejp

Effects of 5-HT_3 receptor-selective agents on locomotor activity in rats following injection into the nucleus accumbens and the ventral tegmental area

Donna M. Gillies [a], Ewan J. Mylecharane [a,*], David M. Jackson [b]

[a] *Department of Pharmacology, University of Sydney, Sydney NSW 2006, Australia*
[b] *Department of Behavioural Pharmacology, Astra Arcus AB, S-151 85 Södertälje, Sweden*

Received 7 December 1995; accepted 5 January 1996

Abstract

5-Hydroxytryptamine (5-HT) is involved in the modulation of dopaminergic activity in the mesolimbic system, but its sites of action and the receptors involved are not well understood. Locomotor activity responses in rats were monitored in Animex automated activity boxes following injection of 5-HT_3 receptor-selective agents directly into two mesolimbic nuclei, the nucleus accumbens and the ventral tegmental area, via stereotactically implanted injection guide cannulae. Neither spontaneous nor dexamphetamine-stimulated locomotor activity was changed by bilateral intra-nucleus accumbens injection of the selective agonist 2-methyl-5-HT or the selective antagonists ondansetron or granisetron. In contrast, intra-ventral tegmental area injection of 2-methyl-5-HT produced significant long-lasting (approximately 240 min) increases in locomotor activity; intra-ventral tegmental area injection of ondansetron elicited an initial inhibition of spontaneous and dexamphetamine-stimulated locomotor activity (for the 0–30 min period), but granisetron had no effect. The hyperlocomotor response to intra-ventral tegmental area 2-methyl-5-HT was abolished by pretreatment with the catecholamine synthesis inhibitor α-methyl-*p*-tyrosine, or by pretreatment with ondansetron. Methiothepin pretreatment had no effect on the hyperlocomotor response to 2-methyl-5-HT, although methiothepin itself produced an initial increase in spontaneous locomotor activity (for the 60–120 min period). Intra-ventral tegmental area injection of 5-carboxamidotryptamine, α-methyl-5-HT or renzapride produced no changes in spontaneous locomotor activity. In some of the ventral tegmental area experiments, other behaviours were also monitored. 2-Methyl-5-HT produced forward locomotion, rearing, and increased wakefulness, but did not appreciably alter circling, grooming or sniffing. Ondansetron alone had no effect on any of these behaviours, but it opposed the 2-methyl-5-HT-induced changes. Methiothepin alone increased forward locomotion and wakefulness but did not alter the other behaviours; it had no effect on the responses to 2-methyl-5-HT. These observations show that 5-HT_3 receptors may mediate increased locomotor activity by modulating firing of mesolimbic dopaminergic cell bodies in the ventral tegmental area rather than terminals in the nucleus accumbens.

Keywords: Mesolimbic system, rat; Nucleus accumbens; Ventral tegmental area; 5-HT_3 receptor; Dopaminergic modulation; Locomotor activity

1. Introduction

Mesolimbic dopaminergic locomotor activity in rodents has proved to be a useful empirical model for identifying drugs with antischizophrenic properties. Neuroleptic dopamine receptor antagonists, however, are associated with adverse extrapyramidal motor effects, because of their blocking actions in other dopaminergic systems, thus there is currently much interest in the development of antischizophrenic drugs with alternative mechanisms of action.

Considerable evidence has been advanced for a regulatory role of 5-hydroxytryptamine (5-HT) on motor activity in rodents, based largely on the presence of a serotoninergic innervation of the mesolimbic system (Azmitia, 1978; Hervé et al., 1987), and the actions of 5-HT and 5-HT receptor antagonists such as methysergide in the nucleus accumbens, a major terminal region in the mesolimbic

* Corresponding author. Tel.: 61-2-351 6202; fax: 61-2-351 3868.

PII S0014-2999(96)00028-3

system (Carter and Pycock, 1978; Costall et al., 1979; Makanjuola et al., 1980; Jones et al., 1981). Some of the effects, however, appear to be contradictory. For example, in some studies, intra-nucleus accumbens injection of 5-HT in rats inhibited the increases in locomotor activity produced by systemic dexamphetamine-induced release of nucleus accumbens dopamine and by direct intra-nucleus accumbens injection of dopamine, and also inhibited spontaneous locomotor activity (Carter and Pycock, 1978; Costall et al., 1979; Jones et al., 1981), but in other studies in rats, intra-nucleus accumbens 5-HT has been reported to have no effect on spontaneous locomotor activity (Jackson et al., 1975; Pijnenburg et al., 1975) or to produce hyperlocomotion (Makanjuola et al., 1980). Intra-nucleus accumbens injection of methysergide has been reported as either enhancing (Carter and Pycock, 1978) or inhibiting (Jones et al., 1981) the hyperlocomotor effect of intra-nucleus accumbens injection of dopamine in rats, but has no effect on spontaneous or dexamphetamine-stimulated locomotor activity (Carter and Pycock, 1978; Costall et al., 1979).

Dopamine metabolite levels and turnover in the ventral tegmental area of the mesolimbic system, which contains dopaminergic cell bodies and dendrites, are considerably less than those in the dopaminergic terminals in the nucleus accumbens (Beart and Gundlach, 1980). Nevertheless, a regulatory action of 5-HT in the ventral tegmental area was suggested by Beart and McDonald (1982), who showed that 5-HT could release dopamine from rat ventral tegmental area slice preparations, and that this action of 5-HT was attenuated by methysergide but not by cyproheptadine.

It is therefore not surprising that the site(s) and receptor mechanisms involved in the modulatory effects of 5-HT on motor activity have not been well understood. Recently, however, there has been a renewal of interest in the role of 5-HT in the mesolimbic system, as a consequence of the development of a comprehensive 5-HT receptor classification scheme (Bradley et al., 1986; Hoyer et al., 1994), the advent of selective 5-HT receptor agonists and antagonists, and the therapeutic potential of alternative antischizophrenic drugs.

Recent data have shown that activation of 5-HT_3 receptors results in an increase in dopaminergic activity in the mesolimbic system in rats, as measured by behavioural, neurochemical and electrophysiological techniques (Hagan et al., 1987, 1990; Carboni et al., 1989a, b; Sorensen et al., 1989; Jiang et al., 1990a, b; Minabe et al., 1991a, b, 1992; Rasmussen et al., 1991; Prisco et al., 1992; Volonté et al., 1992; Pei et al., 1993). This suggests the possibility that 5-HT_3 receptor antagonists may have a selective inhibitory effect on mesolimbic dopaminergic function, and may prove to be effective in disorders such as schizophrenia but without the typical adverse effects produced by dopamine receptor antagonists. In each of these cases, the site of the modulatory action of the 5-HT_3 receptor-selective agents was not able to be identified, as the drugs were administered systemically or i.c.v.

Costall et al. (1987) were able to identify a nucleus accumbens 5-HT_3 receptor mechanism which mediated increased mesolimbic dopaminergic activity, in behavioural studies using rats and marmosets. The increase in locomotor activity produced by intra-nucleus accumbens injection of dexamphetamine in rats was enhanced by intra-nucleus accumbens injection of the 5-HT_3 receptor-selective agonist 2-methyl-5-HT. Intra-nucleus accumbens ondansetron, a 5-HT_3 receptor-selective antagonist, blocked this effect of 2-methyl-5-HT, and when used alone, ondansetron (intra-nucleus accumbens or systemically) inhibited dexamphetamine-stimulated locomotor activity. Neither agent affected spontaneous locomotor activity. The hyperlocomotion induced by the direct intra-nucleus accumbens infusion of dopamine was also blocked by systemically administered ondansetron in rats and marmosets. Costall et al. (1990) reported that the hyperlocomotor effect of intra-nucleus accumbens dopamine infusion in rats was also blocked by the 5-HT_3 receptor-selective antagonists granisetron, tropisetron and zacopride. Thus the identification of a 5-HT_3 receptor-mediated enhancement of mesolimbic dopamine release raises the possibility that selective 5-HT_3 receptor antagonists may prove to be effective 'atypical' antipsychotic agents without adverse extrapyramidal activity (Costall et al., 1990; Tricklebank, 1989, 1992).

Direct supporting evidence for the findings reported by Costall et al. (1987, 1990) has come from microdialysis studies in awake rats which confirm that nucleus accumbens 5-HT_3 receptors mediate an increase in dopamine release (Chen et al., 1991; Kennett and Blackburn, 1991). Other in vivo studies in rats using microdialysis and electrophysiological techniques, however, have shown that 5-HT_3 receptors in the ventral tegmental area are responsible for enhanced mesolimbic dopaminergic activity (Imperato and Angelucci, 1989; Wang and Jiang, 1990).

The aim of our experiments was first to repeat some of the nucleus accumbens investigations reported by Costall et al. (1987, 1990), and then to extend our studies to include the ventral tegmental area, so that the relative importance of 5-HT_3 receptor-mediated modulation of activity in terminals and cell bodies of mesolimbic dopaminergic neurones could be assessed. We have made a direct comparison between the effects of 5-HT_3 receptor-selective agents on spontaneous and dexamphetamine-stimulated locomotor activity in rats, following injection into the nucleus accumbens or the ventral tegmental area via stereotactically implanted guide cannulae. The results obtained indicate that the ventral tegmental area is the principal location of modulatory 5-HT_3 receptors, thus further experiments were aimed at confirming the mechanisms involved in the ventral tegmental area. This was accomplished by investigating the effects of pretreatment with i.p. α-methyl-*p*-tyrosine (which prevents the synthesis of

new stores of dopamine; Jackson et al., 1975), intra-ventral tegmental area injection of ondansetron, and intra-ventral tegmental area injection of methiothepin (a potent 5-HT_1/5-HT_2 receptor antagonist; Bradley et al., 1986; Hoyer et al., 1994), on the hyperlocomotor response to 2-methyl-5-HT. Also, the effects of intra-ventral tegmental area injections of other agents with selective agonist activity at 5-HT receptors (Bradley et al., 1986; Hoyer et al., 1994) were tested: 5-carboxamidotryptamine (5-CT; 5-HT_1-selective), α-methyl-5-HT (5-HT_2-selective), and renzapride (5-HT_3 receptor antagonist/5-HT_4 receptor agonist). Preliminary results have been presented at a meeting of the Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists (Gillies et al., 1991, 1992).

2. Materials and methods

2.1. General

Male Sprague-Dawley rats weighing 240–340 g (University of Sydney Laboratory Animal Services) were used in all experiments. The animals were not habituated to the locomotor activity boxes, and each animal was used only once. Following surgery, the rats were housed in groups of three in a room with a regulated lighting cycle (06:00–18:00 h light), at a constant temperature of 22 ± 2°C. Free access to food and water was allowed except during experimental procedures.

2.2. Surgical implantation of intracerebral injection guide cannulae

On the day of surgery, each rat was pretreated with atropine sulphate (0.8 mg/kg i.p.), followed 20–30 min later by pentobarbitone sodium (48 mg/kg i.p.) to induce anaesthesia. Body temperature was maintained at 37°C via feedback from a rectal probe (Harvard Homeothermic Blanket System). The head was then positioned horizontally in a stereotaxic apparatus (Kopf Instruments) and the upper surface was shaved. A midline incision approximately 5 cm in length was made, and the bregma was exposed. The co-ordinates where the injection guide cannulae were to be placed bilaterally, based on those of Paxinos and Watson (1986), were then marked: for nucleus accumbens injections, 1.6 mm anterior to the bregma and 1.5 mm lateral to the midline; for ventral tegmental area injections, 5.8 mm posterior to the bregma and 0.5 mm lateral to the midline. Holes (1 mm diameter) were then made with a dental drill at the fixed co-ordinates, and two extra holes were drilled to secure supporting screws. The cannulae were positioned over the appropriate holes with a stereotaxic holder and lowered 0.8 mm below the exterior of the skull, so as to rest directly on the brain surface. Dental cement was poured into the incision and left to set. Experimental procedures took place on a randomized basis 3–6 days after recovery from surgery and anaesthesia.

2.3. Measurement of locomotor activity

Rats were wrapped securely in a soft cloth while the guide cannulae were cleared with a 26 gauge needle. Drug or saline (0.9% w/v NaCl) control solutions were then injected into the appropriate nuclei by using injection needles which projected 7.0 or 8.0 mm below the brain surface, into the nucleus accumbens or the ventral tegmental area, respectively. Injections into the nucleus accumbens or the ventral tegmental area were given bilaterally, in a volume of 1 μl/side over a 30 s period, with a further 30 s allowed for drug diffusion before the injection needle was removed. The bilateral intra-nucleus accumbens and intra-ventral tegmental area doses are always expressed in terms of amounts per side. In the experiments in which dexamphetamine-stimulated locomotor activity was monitored, the rats received injections of saline or dexamphetamine at a dose level of either 20 μg/side intra-nucleus accumbens 30 min after the intra-nucleus accumbens injection of test agent or saline (or 5 min after raclopride), or 1.25 mg/kg i.p. 30 min after the intra-ventral tegmental area injection of test agent or saline. It was not practicable to implant both nucleus accumbens and ventral tegmental area guide cannulae in the same animal for intra-nucleus accumbens administration of dexamphetamine in conjunction with intra-ventral tegmental area test agents. In the α-methyl-*p*-tyrosine pretreatment experiments, the pretreatment agent or saline was administered i.p. in two equal doses, 90 min prior to, and immediately before, the intra-ventral tegmental area injection of 2-methyl-5-HT or saline, whereas in the ondansetron and methiothepin pretreatment experiments, the pretreatment agent or saline was administered into the ventral tegmental area 30 min prior to intra-ventral tegmental area 2-methyl-5-HT or saline.

After the final injection, the rats were immediately placed individually in automated activity boxes (LKB Farad Animex). Each activity box comprised a clear acrylic box (450 mm × 330 mm × 180 mm in height), resting on 6 external sensor units, housed in a wooden casing. The boxes were illuminated and maintained at 22 ± 2°C. Oscillator circuits in the external sensors generated a vertical electrical field; the sensitivity of the meter was adjusted so that it recorded the number of times the field was disrupted only by gross movements, such as locomotion in any direction and rearing. In the experiments comparing the nucleus accumbens and the ventral tegmental area effects of 5-HT_3 receptor-selective agents on spontaneous and dexamphetamine-stimulated locomotor activity, monitoring continued for 240 min after the final injection, whereas in the experiments aimed at confirming the mechanisms involved in the ventral tegmental area, monitoring continued for 360 min.

Following the experimental procedure, the rats were killed by decapitation and the brains examined histologically, using toluidine blue stain, to verify the site of injection.

2.4. Observed behaviours

In addition to measurement of automated locomotor activity, behaviours in the ventral tegmental area 2-methyl-5-HT, ondansetron and methiothepin experiments were directly observed. The directly observed behaviours were monitored for a total of 6 periods of 15 min at hourly intervals, commencing 15 min after the rats were placed in the Animex boxes, and continuing until 5 h 30 min after their placement in the boxes. During each 15 min observation period, each of the 3 rats was observed in turn for 5 s every 15 s. If a particular behaviour was present during a 5 s observation, it was scored as a single occurrence of that behaviour. The behaviours scored were forward locomotion (as distinct from locomotion in other directions), rearing (i.e. upright on hind legs), sniffing (i.e. nostrils twitching concurrently with head movements), circling (i.e. turning the body through 360°), grooming (i.e. forepaws wiping the face, or licking, biting or scratching the body), immobility with eyes closed (apparently 'asleep'), and eyes open while remaining immobile (apparently 'awake'). The immobile behaviours were only able to be scored as occurring in the absence of the active behaviours. However, each of the active behaviours rated a score if they occurred at any time during a 5 s observation. Total scores for these behaviours in each of the hourly observation periods were determined by totalling the number of times each behaviour was scored as occurring.

2.5. Drugs

The following drugs were used: atropine sulphate (Boehringer Ingelheim, Germany); pentobarbitone sodium (Abbott, Australia); dexamphetamine sulphate (Fauldings, Australia); raclopride tartrate (Astra, Sweden); 2-methyl-5-HT maleate (Research Biochemicals, USA); ondansetron HCl (Glaxo, UK); granisetron HCl (Beecham, UK); α-methyl-*p*-tyrosine methyl ester HCl (Sigma, USA); methiothepin maleate (Hoffman-La Roche, USA); 5-carboxamidotryptamine maleate (Glaxo, UK); α-methyl-5-HT creatinine sulphate (Wellcome, UK); renzapride HCl (Beecham, UK). All doses are expressed in terms of the salts listed. Drug solutions were freshly prepared in distilled water.

2.6. Statistical analysis

Raw data from the Animex meters, and scores from the observed behaviour experiments, were analysed by 1-factor (or 2-factor where a pretreatment was used) repeated measures (at 60 min intervals, except where indicated otherwise) analysis of variance (SYSTAT, version 3.1, 1986). Where a significant difference was shown ($P < 0.05$), point to point comparisons of mean ± S.E.M. values were made using Student's *t*-test (2-tailed).

3. Results

3.1. Comparison of the effects of injection of 5-HT$_3$ receptor-selective agents into the nucleus accumbens and the ventral tegmental area

The intra-nucleus accumbens injection of dexamphetamine (20 μg/side) produced substantial increases in locomotor activity, compared with spontaneous levels (see Fig. 1). This dexamphetamine-stimulated increase was significantly reduced following pretreatment 5 min beforehand with intra-nucleus accumbens injection of the selective dopamine D_2 receptor antagonist raclopride (5 μg/side; at 30 min after commencing monitoring, mean ± S.E.M. dexamphetamine-stimulated locomotor activity counts were 295 ± 28 and 147 ± 30 in the absence and presence, respectively, of raclopride; $n = 7$ in each group). No significant changes in spontaneous or dexamphetamine-stimulated locomotor activity were produced by the intra-nucleus accumbens injection of 2-methyl-5-HT, at doses of 1, 10 and 100 ng/side, or 10 and 100 ng/side, respectively. Likewise, no significant changes in spontaneous or dexamphetamine-stimulated locomotor activity were produced by ondansetron (0.1, 1, 10 and 100 ng/side, or 1 and 10 ng/side, respectively) or granisetron (1, 10, 100 and 1000 ng/side, or 10 and 100 ng/side, respectively). Fig. 1 shows total spontaneous and dexam-

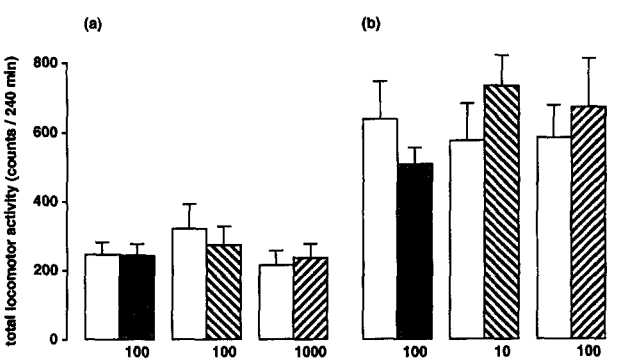

Fig. 1. (a) Total locomotor activity counts, and (b) total dexamphetamine-stimulated locomotor activity counts, in rats receiving intra-nucleus accumbens injections of saline (open columns), 2-methyl-5-HT (stippled columns), ondansetron (right oblique hatched columns) or granisetron (left oblique hatched columns) at the indicated dose levels (ng/side). Dexamphetamine was injected intra-nucleus accumbens (20 μg/side) 30 min after intra-nucleus accumbens saline or test agent. Locomotor activity counts were monitored for the 240 min period immediately following the final intra-nucleus accumbens injection. Columns represent mean values; bars represent S.E.M; $n = 6–8$ for each treatment group. The 5-HT$_3$ receptor-selective agents produced no significant changes (analysis of variance, with repeated measures at 30 min intervals; $P > 0.05$).

phetamine-stimulated locomotor activity counts over the 240 min monitoring period in these experiments for the highest doses of 2-methyl-5-HT, ondansetron and granisetron used.

Intra-ventral tegmental area injection of 2-methyl-5-HT (1 and 10 ng/side) produced no significant increases in spontaneous locomotor activity, but the injection of 100 ng/side resulted in significant hyperlocomotion. Total spontaneous locomotor activity counts over the 240 min monitoring period in these experiments are shown in Fig. 2 for these three doses of 2-methyl-5-HT. The effect of the 100 ng/side dose was clearly maintained until the end of the 240 min recording period. Therefore, in the subsequent series of experiments investigating the mechanism of the hyperlocomotor action of intra-ventral tegmental area injection of 2-methyl-5-HT (see section 3.2 below), it was decided to increase the monitoring period to 360 min, and to use a higher dose (500 ng/side) to ensure that pretreatments were able to produce unequivocal effects. For comparison, total spontaneous locomotor activity counts (over the first 240 min of the monitoring period) from the latter experiments are also shown in Fig. 2. In these experiments, the onset of the hyperlocomotion produced by the 100 and 500 ng/side doses of 2-methyl-5-HT appeared to be between 60 and 120 min after its injection. When all four doses of 2-methyl-5-HT were injected near to but not into the ventral tegmental area, there were no significant changes in locomotor activity (see Fig. 2).

Ondansetron, at all intra-ventral tegmental area injection dose levels tested (1, 10 and 100 ng/side), produced significant decreases of similar magnitude in spontaneous locomotor activity for the first 30 min of the 240 min monitoring period, but in the remaining period, locomotor activity was similar to that in the corresponding saline control experiments. No significant changes were produced

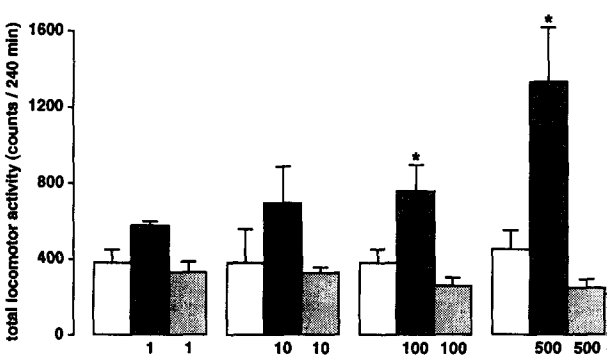

Fig. 2. Total locomotor activity counts in rats receiving intra-ventral tegmental area injections of saline (open columns) or 2-methyl-5-HT (dark stippled columns) at the indicated doses (ng/side), or injections of the same doses of 2-methyl-5-HT near to but not into the ventral tegmental area (light stippled columns). Locomotor activity counts were monitored for the 240 min period immediately following injection. Columns represent mean values; bars represent S.E.M; $n = 7–10$ for each treatment group; * significant 2-methyl-5-HT-induced difference from the corresponding saline control value (analysis of variance, with repeated measures at 30 min intervals; $P < 0.05$).

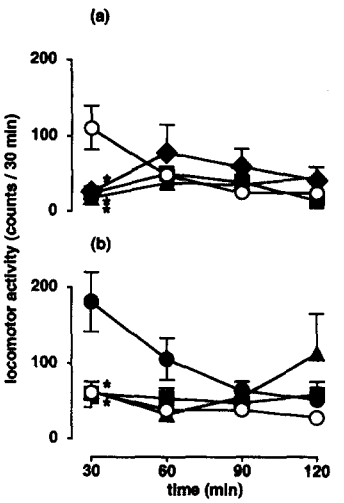

Fig. 3. (a) Locomotor activity counts/30 min in rats receiving intra-ventral tegmental area injections of saline (○) or ondansetron at doses of 1 (▲), 10 (■) or 100 (◆) ng/side; and (b) dexamphetamine-stimulated locomotor activity counts/30 min in rats receiving intra-ventral tegmental area injections of saline (●) or ondansetron at doses of 1 (▲) or 10 (■) ng/side. Dexamphetamine was injected i.p. (1.25 mg/kg) 30 min after intra-ventral tegmental area saline or test agent. The locomotor activity counts plotted were those monitored in the 120 min period immediately following the final intra-ventral tegmental area or i.p. injection. Points represent mean values; bars represent S.E.M.; $n = 6–9$ for each treatment group. Dexamphetamine produced a significant increase in locomotor activity (analysis of variance, with repeated measures at 30 min intervals; $P < 0.05$) compared with a further control group receiving intra-ventral tegmental area and i.p. saline only (data plotted on same axes; ○). All doses of ondansetron produced significant decreases in both spontaneous and dexamphetamine-stimulated locomotor activity (analysis of variance, with repeated measures at 30 min intervals; $P < 0.05$); * significant ondansetron-induced difference from the corresponding saline control value (t-test; $P < 0.05$).

when these doses of ondansetron were injected near to but not into the ventral tegmental area. Fig. 3a shows the mean locomotor activity counts/30 min for the 120 min period immediately following intra-ventral tegmental area ondansetron; these values were significantly lower than those in the corresponding saline control experiments only for the first 30 min period following injection, when spontaneous activity levels were at their highest. In contrast, intra-ventral tegmental area injection of granisetron produced no significant changes in spontaneous locomotor activity during the 240 min monitoring period at any of the dose levels tested (1, 10 and 100 ng/side; data not shown). Injections of these doses of ondansetron or granisetron near to but not into the ventral tegmental area were without effect (data not shown).

Dexamphetamine (1.25 mg/kg i.p.) produced significant increases in locomotor activity in comparison with spontaneous levels (see Fig. 3b). As was observed for spontaneous locomotor activity, for the first 30 min of the 240 min monitoring period, intra-ventral tegmental area injection of ondansetron produced significant decreases of similar magnitude in dexamphetamine-stimulated locomo-

tor activity, at both doses tested (1 and 10 ng/side; Fig. 3b). Ondansetron injected at these doses near to but not into the ventral tegmental area was without effect, as was injection of granisetron either into or near to the ventral tegmental area (100 and 1000 ng/side; data not shown).

3.2. Mechanisms of the hyperlocomotor effect of injection of 2-methyl-5-HT into the ventral tegmental area

Intra-ventral tegmental area injection of 2-methyl-5-HT (500 ng/side) caused a significant increase in spontaneous locomotor activity, peaking at 180 min and lasting for approximately 240 min, as illustrated in Figs. 4–6, which show mean locomotor activity counts/60 min over the 360 min monitoring period. Pretreatment with α-methyl-*p*-tyrosine (200 mg/kg i.p. doses 90 min prior to, and immediately before, injection of 2-methyl-5-HT) completely abolished this hyperlocomotion (Fig. 4), while treatment with α-methyl-*p*-tyrosine alone did not significantly affect spontaneous locomotor activity. Ondansetron pretreatment (100 ng/side intra-ventral tegmental area injection) abolished 2-methyl-5-HT-induced hyperlocomotion, but when used alone it had no significant effect on spontaneous locomotor activity (Fig. 5). Intra-ventral tegmental area injection of methiothepin (100 ng/side) had no effect on the hyperlocomotor response to 2-methyl-5-HT; however, it caused an initial increase in spontaneous locomotor activity on its own (for the 60–120 min period; Fig. 6). Injections of the other 5-HT receptor-selective agents into the ventral tegmental area (5-CT, 30 ng/side;

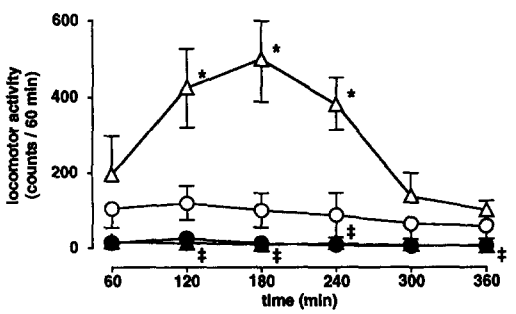

Fig. 4. Locomotor activity counts/60 min in rats receiving i.p injections of saline pretreatment followed by intra-ventral tegmental area injection of saline (○), i.p. injections of saline pretreatment followed by intra-ventral tegmental area injection of 2-methyl-5-HT (500 ng/side; △), i.p. injections of α-methyl-*p*-tyrosine pretreatment (400 mg/kg) followed by intra-ventral tegmental area injection of saline (●), or i.p. injections of α-methyl-*p*-tyrosine pretreatment (400 mg/kg) followed by intra-ventral tegmental area injection of 2-methyl-5-HT (500 ng/side; ▲). The i.p. saline or α-methyl-*p*-tyrosine pretreatments were administerd in two equal doses, 90 min prior to, and immediately before the intra-ventral tegmental area 2-methyl-5-HT or saline and the commencement of locomotor activity monitoring. Locomotor activity was monitored for the 360 min period immediately following the final injection. Points represent mean values; bars represent S.E.M.; $n = 6$–8 for each treatment group. 2-Methyl-5-HT alone produced a significant increase in spontaneous locomotor activity (analysis of variance; $P < 0.05$); * significant difference from the corresponding saline control value (*t*-test; $P < 0.05$). Pretreatment with α-methyl-*p*-tyrosine had no significant effect on spontaneous locomotor activity (analysis of variance; $P > 0.05$), but it abolished the 2-methyl-5-HT-induced hyperlocomotion (analysis of variance; $P < 0.05$); ‡ significant difference from the corresponding 2-methyl-5-HT alone value (*t*-test; $P < 0.05$).

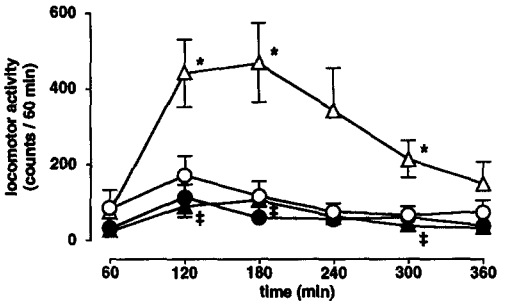

Fig. 5. Locomotor activity counts/60 min in rats receiving intra-ventral tegmental area injections of saline pretreatment followed by saline (○), saline pretreatment followed by 2-methyl-5-HT (500 ng/side; △), ondansetron pretreatment (100 ng/side) followed by saline (●), or ondansetron pretreatment (100 ng/side) followed by 2-methyl-5-HT (500 ng/side; ▲). The intra-ventral tegmental area saline or ondansetron pretreatments were administered 30 min prior to intra-ventral tegmental area 2-methyl-5-HT or saline and the commencement of locomotor activity monitoring. Locomotor activity was monitored for the 360 min period immediately following the final intra-ventral tegmental area injection. Points represent mean values; bars represent S.E.M.; $n = 6$-8 for each treatment group. 2-Methyl-5-HT produced a significant increase in spontaneous locomotor activity (analysis of variance; $P < 0.05$); * significant difference from the corresponding saline control value (*t*-test; $P < 0.05$). Pretreatment with ondansetron had no significant effect on spontaneous locomotor activity (analysis of variance; $P > 0.05$), but it abolished the 2-methyl-5-HT-induced hyperlocomotion (analysis of variance; $P < 0.05$); ‡ significant difference from the corresponding 2-methyl-5-HT alone value (*t*-test; $P < 0.05$).

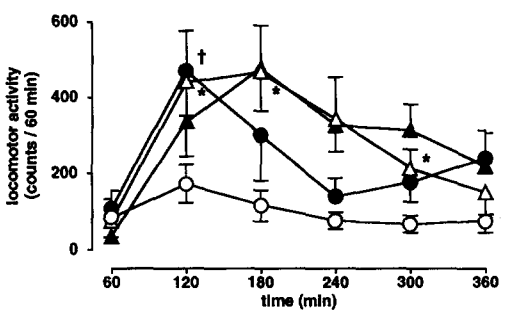

Fig. 6. Locomotor activity counts/60 min in rats receiving intra-ventral tegmental area injections of saline pretreatment followed by saline (○), saline pretreatment followed by 2-methyl-5-HT (500 ng/side; △), methiothepin pretreatment (100 ng/side) followed by saline (●), or methiothepin pretreatment (100 ng/side) followed by 2-methyl-5-HT (500 ng/side; ▲). Pretreatment times and the locomotor activity monitoring period were as described in Fig. 5. Points represent mean values; bars represent S.E.M.; n = 6–8 for each treatment group. 2-Methyl-5-HT produced a significant increase in spontaneous locomotor activity (analysis of variance; $P < 0.05$); * significant difference from the corresponding saline control value (*t*-test; $P < 0.05$). Pretreatment with methiothepin had no significant effect on the 2-methyl-5-HT-induced hyperlocomotion (analysis of variance; $P > 0.05$), but it caused an initial significant increase in spontaneous locomotor activity (analysis of variance; $P < 0.05$); † significant difference from the corresponding saline control value (*t*-test; $P < 0.05$).

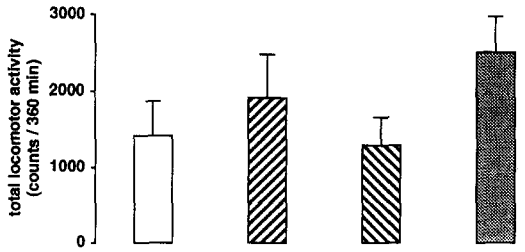

Fig. 7. Total locomotor activity counts in rats receiving intra-ventral tegmental area injections of saline (open column), 5-CT (30 ng/side; left oblique hatched column), α-methyl-5-HT (100 ng/side; right oblique hatched column), or renzapride (100 ng/side; stippled column). Locomotor activity counts were monitored for the 360 min period immediately following intra-ventral tegmental area injection. Columns represent mean values; bars represent S.E.M.; $n = 7–8$ for each treatment group. The 5-HT receptor-selective agents produced no significant changes (analysis of variance; $P > 0.05$).

α-methyl-5-HT, 100 ng/side; renzapride, 100 ng/side) did not produce any significant changes in spontaneous locomotor activity (Fig. 7).

3.3. Effects of injection of 2-methyl-5-HT into the ventral tegmental area on observed behaviours

2-Methyl-5-HT (500 ng/side) significantly increased forward locomotion and rearing, from 120–300 and 120–360 min, respectively, after its intra-ventral tegmental area injection (Fig. 8a,b). Time spent 'asleep' was significantly less than saline controls from 180–360 min (Fig. 8c) while time spent apparently 'awake' but immobile was significantly higher than the control group at 360 min (Fig. 8d). Time spent 'awake' was not significantly higher than that in the saline controls from 180–300 min (and was significantly lower than the saline controls at 120 min), because the active behaviours predominated during this period and therefore 'awake' behaviour was not scored as occurring (see Materials and methods).

Ondansetron pretreatment (100 ng/side intra-ventral tegmental area injection) abolished the 2-methyl-5-HT-induced forward locomotion and rearing behaviours (Fig. 8a,b), and also opposed the increase in wakefulness induced by 2-methyl-5-HT (Fig. 8c,d). Ondansetron alone had no significant effect on any of these behaviours (Fig. 8).

Methiothepin pretreatment (100 ng/side intra-ventral tegmental area injection) had no effect on the 2-methyl-5-HT-induced behaviours, although it was found that methiothepin alone produced an initial significant increase in forward locomotion (for the 60–120 min period) and it significantly increased wakefulness, but it had no effect on rearing (data not shown).

Of the other behaviours observed in these experiments,

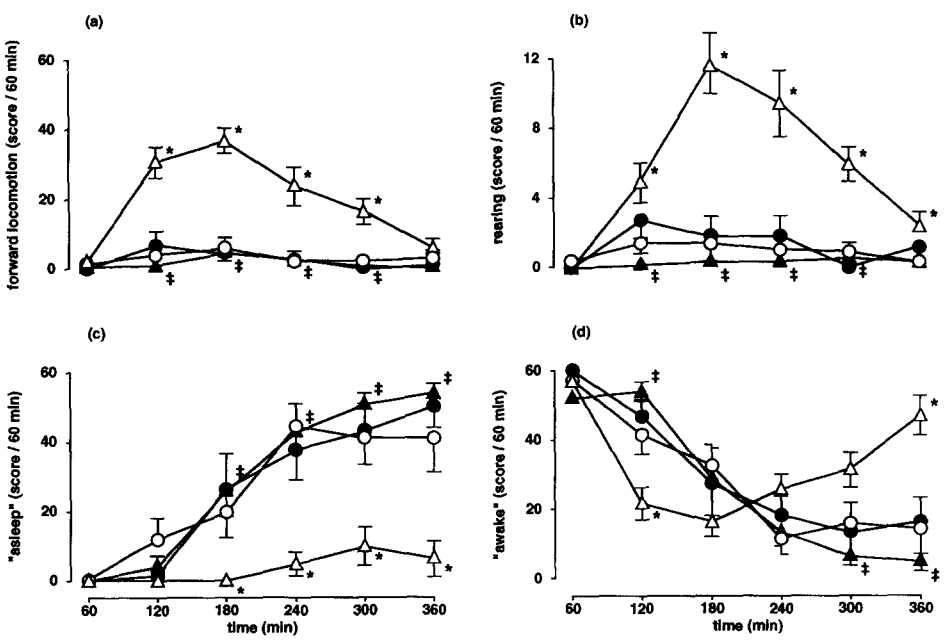

Fig. 8. Observed behaviour scores/60 min for (a) forward locomotion, (b) rearing, (c) apparently 'asleep', and (d) apparently 'awake' but immobile, in rats receiving intra-ventral tegmental area injections of saline pretreatment followed by saline (○), saline pretreatment followed by 2-methyl-5-HT (500 ng/side; △), ondansetron pretreatment (100 ng/side) followed by saline (●), or ondansetron pretreatment (100 ng/side) followed by 2-methyl-5-HT (500 ng/side; ▲). Pretreatment times and the locomotor activity monitoring period were as described in Fig. 5. Points represent mean values; bars represent S.E.M.; $n = 6–8$ for each treatment group. 2-Methyl-5-HT produced significant increases in the forward locomotion, rearing, and 'awake' scores, and significant decreases in the 'asleep' scores (analysis of variance; $P < 0.05$; * significant difference from the corresponding saline control value (*t*-test; $P < 0.05$). In (d), 2-methyl-5-HT also produced a significant decrease in the 'awake' score for the 60–120 min period, but this is due to the significant increase in forward locomotion and rearing at this time which prevents 'awake' but immobile scores being registered. Pretreatment with ondansetron had no significant effect on any of the observed behaviours (analysis of variance; $P > 0.05$), but it abolished the 2-methyl-5-HT-induced effects; ‡ significant difference from the corresponding 2-methyl-5-HT alone value (*t*-test; $P < 0.05$).

circling and grooming were not affected by 2-methyl-5-HT or by ondansetron or methiothepin alone, and pretreatment with the antagonists did not reveal any 2-methyl-5-HT-induced changes (data not shown). The only effect on sniffing was a small but significant increase 240 min after the intra-ventral tegmental area injection of 2-methyl-5-HT, which was not affected by pretreatment with either ondansetron or methiothepin.

4. Discussion

The lack of effect of intra-nucleus accumbens injection of any of the 5-HT_3 receptor-selective agents, and the increases in locomotor activity (as well as forward locomotion and rearing; see below) in response to intra-ventral tegmental area 2-methyl-5-HT, suggest that 5-HT_3 receptors may increase activity in the mesolimbic system by modulating firing of dopaminergic cell bodies in the ventral tegmental area rather than terminals in the nucleus accumbens. The increase in spontaneous locomotor activity caused by 2-methyl-5-HT was dependent on newly synthesized dopamine stores, as indicated by the absence of activity of 2-methyl-5-HT following i.p. pretreatment with α-methyl-*p*-tyrosine.

The observations that injection of 2-methyl-5-HT near to but not into the ventral tegmental area is inactive indicates that its action is not due to diffusion into other nuclei, even though the onset of its effect ranges from 60 to 120 min. The long duration of the hyperlocomotor response to intra-ventral tegmental area injection of 2-methyl-5-HT in our experiments provides further evidence that diffusion away from the site of injection and metabolic degradation is relatively slow.

Our observations of a lack of effect on spontaneous locomotor activity following intra-nucleus accumbens injections of 2-methyl-5-HT and ondansetron are consistent with those of Costall et al. (1987), but our findings on dexamphetamine-stimulated activity differ. The reasons for this are not clear. There are some minor methodological differences, in that the intra-nucleus accumbens dose of dexamphetamine in our experiments was 2-fold higher, and the period between surgery and experimental investigation was shorter. However, the inhibition of the intra-nucleus accumbens dexamphetamine-induced hyperlocomotion by the dopamine D_2 receptor antagonist raclopride in our experiments confirms that the hyperlocomotor effect was dopaminergic, as was the case for Costall et al. (1987) who used sulpiride and fluphenazine. In our dexamphetamine-stimulated locomotor activity experiments, the dose ranges of intra-nucleus accumbens 2-methyl-5-HT (10–100 ng/side) and ondansetron (1–10 ng/side) were comparable to those used by Costall et al. (1987; 0.1–10 ng/side and 0.01–1 ng/side, respectively), in that the lowest dose levels we used were reported to produce significant changes by Costall et al. (1987). It should also be noted that in our experiments, intra-ventral tegmental area administration of 2-methyl-5-HT and ondansetron at similar dose levels (100–500 ng/side, and 1–100 ng/side) did produce significant changes in locomotor activity.

As noted previously, the direct supporting evidence for a dopaminergic modulatory role of 5-HT_3 receptors in the nucleus accumbens comes from microdialysis studies in awake rats (Chen et al., 1991; Kennett and Blackburn, 1991). In these studies, 5-HT_3 receptor-selective agonists were administered directly into the nucleus accumbens via the microdialysis probe, together with 5-HT_3 receptor-selective antagonists administered either directly into the nucleus accumbens, or systemically. Other support for a nucleus accumbens role of 5-HT_3 receptors comes from microdialysis studies in awake rats in which dopamine release induced by perfusion of either ethanol or 5-HT into the nucleus accumbens was inhibited by co-perfusion of 5-HT_3 receptor-selective antagonists directly into the nucleus accumbens (Yoshimoto et al., 1992; Parsons and Justice, 1993). The conclusions which can be drawn from the latter studies are limited, however, because selective 5-HT_3 receptor agonists were not used; in fact, Parsons and Justice (1993) also reported that intra-nucleus accumbens 5-$HT_{1A/1B}$ and 5-HT_2 receptor-selective antagonists produced reductions in the dopamine release response to intra-nucleus accumbens 5-HT. Layer et al. (1992) found that dexamphetamine-stimulated locomotor activity in rats was increased by intra-nucleus accumbens phenylbiguanide, a 5-HT_3 receptor-selective agonist. This finding is not conclusive, however, because the 5-HT_3 receptor-selective antagonist MDL 72222 at the single intra-nucleus accumbens dose used did not block the phenylbiguanide-induced increase in dopamine release. Interestingly, in the studies described by Costall et al. (1987), the doses of intra-nucleus accumbens ondansetron required to antagonize 2-methyl-5-HT-induced hyperlocomotion in a dose-dependent manner (10–1000 ng/side) were much higher than those producing inhibitory effects on hyperlocomotion when used alone (0.01–1 ng/side). This raises the possibility that this inhibitory effect of ondansetron alone in the nucleus accumbens may not be related to 5-HT_3 receptor antagonism.

Our suggestion that ventral tegmental area rather than nucleus accumbens 5-HT_3 receptors modulate locomotor activity was supported by the findings in the present study with intra-ventral tegmental area injections of other selective 5-HT receptor agonists and antagonists. The 2-methyl-5-HT-induced hyperlocomotion was abolished by ondansetron, but was unaffected by methiothepin (5-HT_1/5-HT_2 receptor-selective antagonist). Agents with 5-HT_1, 5-HT_2 and 5-HT_4 receptor-selective agonist actions (5-CT, α-methyl-5-HT, and renzapride, respectively) produced no significant changes in spontaneous locomotor activity. The dose levels at which these agents were used were based on their potencies relative to 2-methyl-5-HT (Hoyer et al., 1994). It therefore seems reasonable to conclude that the

hyperlocomotor action of 2-methyl-5-HT is mediated by 5-HT_3 receptors.

The initial inhibitory effect of intra-ventral tegmental area ondansetron but not granisetron on both spontaneous and dexamphetamine-stimulated locomotor activity in our experiments (see Fig. 3) is difficult to interpret. This effect of ondansetron may reflect an involvement of endogenous 5-HT via a mesolimbic 5-HT_3 receptor mechanism, but the lack of effect of granisetron would argue against this. Taking into account the fact that ondansetron has a 5-HT_3 receptor affinity approximately 2–3-fold less than that of granisetron (Fozard, 1990), it is most unlikely that the doses of granisetron used were too low. It is possible that opposing effects may arise with increasing doses of granisetron, because a loss of efficacy at high doses has been reported in behavioural studies, but nevertheless, exactly the same phenomenon has also been described for ondansetron (Costall et al., 1990; Hagan et al., 1990).

The apparent lack of dose dependency and short duration of this ondansetron-induced inhibition of locomotor activity also make interpretation of these findings complex. A possible explanation is that control spontaneous and dexamphetamine-stimulated locomotor activities were highest during the first 30 min after commencing recording, and that these locomotor activity levels were relatively modest. This would tend to mask any continuing or more substantial inhibition produced by the higher intra-ventral tegmental area doses of ondansetron. Alternatively, the effect of the lowest dose of ondansetron used may well have been maximal.

The inhibitory effect of intra-ventral tegmental area injection of ondansetron alone in the first 30 min after its administration cannot account for its blockade of 2-methyl-5-HT-induced hyperlocomotion, because ondansetron alone did not significantly change locomotor activity at times corresponding to those when 2-methyl-5-HT alone was producing hyperlocomotion. Methiothepin alone, when injected into the ventral tegmental area, produced a significant increase in locomotor activity only for the 60–120 min period during the 360 min of monitoring. Although methiothepin pretreatment had no effect on the response to 2-methyl-5-HT, it could be expected to have added to the hyperlocomotor effect of 500 ng/side of 2-methyl-5-HT during this 60–120 min period. The fact that this was not seen (Fig. 6) could be explained by the dose of 2-methyl-5-HT used being at or close to a maximal level. The mechanism responsible for the hyperlocomotor effect of methiothepin during this 60–120 min period is unknown. It is unlikely to be due to blockade of a 5-HT_1 or 5-HT_2 receptor-mediated inhibition of spontaneous locomotor activity, because intra-ventral tegmental area injection of 5-CT or α-methyl-5-HT produced no changes in locomotor activity.

A 5-HT_3 receptor-mediated modulation of dopaminergic mesolimbic activity is further supported by the observations that intra-ventral tegmental area injection of 2-methyl-5-HT increased behaviours which are generally associated with raised mesolimbic dopaminergic activity, namely forward locomotion and rearing (Makanjuola et al., 1980; Beninger, 1983; Evenden and Ryan, 1988), although mesolimbic dopaminergic activation may not always produce rearing (Jackson et al., 1975). The time spent apparently 'asleep' was decreased significantly by intra-ventral tegmental area injection of 2-methyl-5-HT, while the time spent apparently 'awake' was significantly increased. Although the 2-methyl-5-HT-induced increases in rearing and forward locomotion had almost or completely ceased by 360 min (as had hyperlocomotor activity), marked wakefulness was still present, perhaps reflecting the continuation of more subtle behavioural changes related to alertness. All of these 2-methyl-5-HT-induced behavioural effects were abolished by pretreatment with ondansetron injected into the ventral tegmental area, but were not affected by intra-ventral tegmental area methiothepin, consistent with a 5-HT_3 receptor involvement in these observed behaviours. Ondansetron alone did not affect any of these behaviours. Methiothepin alone, however, caused an increase in forward locomotion and wakefulness for the 60–120 min period during the 360 min of monitoring, by unknown mechanisms, consistent with its hyperlocomotor activity at this time. Interestingly, rearing was not affected by methiothepin alone.

Behaviours generally associated with other dopaminergic systems, i.e. sniffing, circling and grooming (Beninger, 1983; Evenden and Ryan, 1988) were not produced by intra-ventral tegmental area injection of 2-methyl-5-HT, nor by ondansetron or methiothepin alone or in combination with 2-methyl-5-HT, indicating that the mesolimbic dopaminergic system is selectively activated by intra-ventral tegmental area 2-methyl-5-HT. The only exception was a brief 2-methyl-5-HT-induced increase in sniffing behaviour, which was unaffected by ondansetron or methiothepin pretreatment. It should be noted, however, that sniffing behaviour can also be elicited by mesolimbic activation with systemic or intra-nucleus accumbens injection of apomorphine, or by intra-nucleus accumbens injection of dopamine (Costall et al., 1975; Makanjuola et al., 1980), thus activation of specific mesolimbic pathways associated with sniffing behaviour may not involve 5-HT_3 receptor mechanisms.

Our conclusion that 5-HT_3 receptors in the ventral tegmental area mediate hyperlocomotion in rats is consistent with findings by other investigators in microdialysis and electrophysiological studies. As noted previously, Imperato and Angelucci (1989) measured nucleus accumbens dopamine and its metabolites in awake freely moving rats using microinfusion probes, and found that both systemic and intra-ventral tegmental area injection of the 5-HT_3 receptor-selective antagonist tropisetron inhibited the release of dopamine in the nucleus accumbens produced by systemic morphine. When injected into the nucleus accumbens, however, tropisetron was without effect. Wang and

Jiang (1990) reported that iontophoretic administration of 2-methyl-5-HT resulted in the activation of ventral tegmental area dopamine neurones in anaesthetized rats; this excitatory effect of 2-methyl-5-HT was blocked by the 5-HT_3 receptor-selective antagonists granisetron and tropisetron, but was unaffected by antagonists with selective actions at 5-HT_{1D} and 5-HT_2 receptors (metergoline, ritanserin).

Other authors have reported that 5-HT can release dopamine from rat ventral tegmental area slices in vitro (Beart and McDonald, 1982), and intra-ventral tegmental area administration of 5-HT in awake rats results in elevated levels of dopamine metabolites (but not dopamine) in the nucleus accumbens, as detected by microinfusion probes (Guan and McBride, 1989). In these studies, methysergide but not cyproheptadine antagonized the effect of 5-HT in the rat ventral tegmental area slice preparations, and the 5-HT receptor agonist trifluoromethylphenylpiperazine but not 8-hydroxy-2-(di-*n*-propylamino)tetralin mimicked the effect of 5-HT in the microinfusion study. This might suggest the involvement of 5-HT_1 and/or 5-HT_2 receptor mechanisms in 5-HT-induced ventral tegmental area dopaminergic activation, but these findings are not conclusive, due to the lack of selectivity of methysergide and trifluoromethylphenylpiperazine at 5-HT_1 and 5-HT_2 receptor subtypes (Hoyer et al., 1994).

Although it has been reported that there are significant densities of 5-HT_3 binding sites in the rat nucleus accumbens/olfactory tubercle (Kilpatrick et al., 1987), in autoradiographic studies in rat brain, nucleus accumbens 5-HT_3 binding site levels were relatively low in comparison with other areas such as amygdaloid nuclei, the nucleus tractus solitarii and the dorsal motor nucleus of the vagus (Barnes et al., 1990; Perry, 1990; Gehlert et al., 1991; Laporte et al., 1992). In some autoradiographic studies, 5-HT_3 binding site densities in rat ventral tegmental area have been reported to be low or non-detectable (Barnes et al., 1990; Laporte et al., 1992), although Perry (1990) found that 5-HT_3 binding site levels are higher in the ventral tegmental area than in the nucleus accumbens. Regardless of the relative levels of nucleus accumbens and ventral tegmental area 5-HT_3 binding site densities, it may well be that very small numbers of 5-HT_3 receptors in either nucleus may still be able to exert profound modulatory effects on dopamine release.

The findings that 5-HT_3 receptors may have a modulatory role in mesolimbic dopaminergic function provide a basis for the premise that 5-HT_3 receptor antagonists may prove to have a useful therapeutic effect in schizophrenia, without the disadvantages of the neuroleptic drugs currently available (Tricklebank, 1989, 1992). To date, there have been few clinical investigations, and the results reported are equivocal. Ondansetron has been found to be effective in schizophrenia without producing extrapyramidal effects, in two uncontrolled studies (White et al., 1991; DeVeaugh-Geiss et al., 1992). It was also effective in attenuating amphetamine-induced anorexia (Silverstone et al., 1992) and controlling levodopa-induced hallucinosis in patients with Parkinson's disease (Zoldan et al., 1993). In a placebo-controlled trial in schizophrenic patients, however, results with ondansetron were inconclusive in that overall effects were not significantly different from placebo, although some measures suggested improvement (Meltzer, 1991; McBain et al., 1992). Zacopride produced no significant improvement in schizophrenia in a placebo-controlled trial (Newcomer et al., 1992).

The results of the present investigation add weight to the increasing body of evidence that 5-HT_3 receptors may have an important modulatory role in the mesolimbic dopaminergic system. Our conclusion is that a 5-HT_3 receptor-mediated hyperlocomotor effect in rats involves the modulation of firing of mesolimbic dopaminergic cell bodies in the ventral tegmental area rather than terminals in the nucleus accumbens. Whether or not these findings can be exploited to provide a useful means of treating schizophrenia remains to be established.

Acknowledgements

This project was supported by the Australian Research Council. The authors wish to thank Astra (Sweden), Glaxo (UK), Beecham (UK), Hoffman-La Roche (USA) and Wellcome (UK) for gifts of drugs.

References

Azmitia, E.C., 1978, The serotonin-producing neurons of the midbrain medial and dorsal raphe nuclei, in: Handbook of Psychopharmacology, Vol. 9, Chemical Pathways in the Brain, ed. L.L. Iversen, S.D. Iversen and S.H. Snyder (Plenum Press, New York) p. 233.

Barnes, J.M., N.M. Barnes, S. Champaneria, B. Costall and R.J. Naylor, 1990, Characterisation and autoradiographic localisation of 5-HT_3 recognition sites identified with [^{3}H]-(*S*)-zacopride in the forebrain of the rat, Neuropharmacology 29, 1037.

Beart, P.M. and A.L. Gundlach, 1980, 3,4-Dihydroxyphenylacetic acid (DOPAC) and the rat mesolimbic dopaminergic pathway: drug effects and evidence for somatodendritic mechanisms, Br. J. Pharmacol. 69, 241.

Beart, P.M. and D. McDonald, 1982, 5-Hydroxytryptamine and 5-hydroxytryptaminergic-dopaminergic interactions in the ventral tegmental area of rat brain, J. Pharm. Pharmacol. 34, 591.

Beninger, R.J., 1983, The role of dopamine in locomotor activity and learning, Brain Res. Rev. 6, 173.

Bradley, P.B., G. Engel, W. Feniuk, J.R. Fozard, P.P.A. Humphrey, D.N. Middlemiss, E.J. Mylecharane, B.P. Richardson and P.R. Saxena, 1986, Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine, Neuropharmacology 25, 563.

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

Carboni, E., E. Acquas, P. Leone and G. Di Chiara, 1989b, 5-HT_3 receptor antagonists block morphine- and nicotine- but not amphetamine-induced reward, Psychopharmacology 97, 175.

Carter, C.J. and C.J. Pycock, 1978, Differential effects of central sero-

tonin manipulation on hyperactive and stereotyped behaviour, Life Sci. 23, 953.

Chen, J., H.M. Van Praag and E.L. Gardner, 1991, Activation of 5-HT_3 receptor by 1-phenylbiguanide increases dopamine release in the rat nucleus accumbens, Brain Res. 543, 354.

Costall, B., R.J. Naylor and J.L. Neumeyer, 1975, Differences in the nature of the stereotyped behaviour induced by aporphine derivatives in the rat and in their actions in extrapyramidal and mesolimbic brain areas, Eur. J. Pharmacol. 31, 1.

Costall, B., S.-C.G. Hui and R.J. Naylor, 1979, The importance of serotonergic mechanisms for the induction of hyperactivity by amphetamine and its antagonism by intra-accumbens (3,4-dihydroxyphenylamino)-2-imidazoline (DPI), Neuropharmacology 18, 605.

Costall, B., A.M. Domeney, R.J. Naylor and M.B. Tyers, 1987, Effects of the 5-HT_3 receptor antagonist, GR38032F, on raised dopaminergic activity in the mesolimbic system of the rat and marmoset brain, Br. J. Pharmacol. 92, 881.

Costall, B., A.M. Domeny and R.J. Naylor, 1990, 5-HT_3 receptor antagonists attenuate dopamine-induced hyperactivity in the rat, NeuroReport 1, 77.

DeVeaugh-Geiss, J., S. McBain, P. Cooksey and J.M. Bell, 1992, The effects of a novel 5-HT_3 antagonist, ondansetron, in schizophrenia: results from uncontrolled trials, in: Novel Antipsychotic Drugs, ed. H.Y. Meltzer (Raven Press, New York) p. 225.

Evenden, J.L. and C.N. Ryan, 1988, Behavioral responses to psychomotor stimulant drugs: localization in the central nervous system, Pharmacol. Ther. 36, 151.

Fozard, J.R., 1990, Agonists and antagonists of 5-HT_3 receptors, in: Cardiovascular Pharmacology of 5-Hydroxytryptamine: Prospective Therapeutic Applications, eds. P.R. Saxena, D.I. Wallis, W. Wouters and P. Bevan (Kluwer, Dordrecht) p. 101.

Gehlert, D.R., S.L. Gackenheimer, D.T. Wong and D.W. Robertson, 1991, Localization of 5-HT_3 receptors in the rat brain using [^{3}H]LY278584, Brain Res. 553, 149.

Gillies, D.M., E.J. Mylecharane and D.M. Jackson, 1991, 5-HT_3-receptor-mediated modulation of mesolimbic locomotor activity in rats, Clin. Exp. Pharmacol. Physiol. Suppl. 18, 20.

Gillies, D.M., E.J. Mylecharane and D.M. Jackson, 1992, Modulation of mesolimbic locomotor activity in rats by 5-HT_3 receptors in the ventral tegmental area, Clin. Exp. Pharmacol. Physiol. Suppl. 21, 23.

Guan, X.-M. and W.J. McBride, 1989, Serotonin microinfusion into the ventral tegmental area increses accumbens dopamine release, Brain Res. Bull. 23, 541.

Hagan, R.M., A. Butler, J.M. Hill, C.C. Jordan, S.J. Ireland and M.B. Tyers, 1987, Effect of the 5-HT_3 receptor antagonist, GR38032F, on responses to injection of a neurokinin agonist into the ventral tegmental area of the rat brain, Eur. J. Pharmacol. 138, 303.

Hagan, R.M., B.J. Jones, C.C. Jordan and M.B. Tyers, 1990, Effect of 5-HT_3 receptor antagonists on responses to selective activation of mesolimbic dopaminergic pathways in the rat, Br. J. Pharmacol. 99, 227.

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.

Hoyer, D., D.E. Clarke, J.R. Fozard, P.R. Hartig, G.R. Martin, E.J. Mylecharane, P.R. Saxena and P.P.A. Humphrey, 1994, International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin), Pharmacol. Rev. 46, 157.

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.

Jackson, D.M., N.-E. Andén and A. Dahlström, 1975, A functional effect of dopamine in the nucleus accumbens and in some other dopamine-rich parts of the rat brain, Psychopharmacologia (Berlin) 45, 139.

Jiang, L.H., C.R. Ashby Jr., R.J. Kasser and R.Y. Wang, 1990a, The effect of intraventricular administration of the 5-HT_3 receptor agonist 2-methylserotonin on the release of dopamine in the nucleus accumbens: an in vivo chronocoulometric study, Brain Res. 513, 156.

Jiang, L.H., L. Fishkin, S.X. Tian and R.Y. Wang, 1990b, 2-Me-5-HT-induced DA release in the nucleus accumbens mediated by the 5-HT_3 receptors, Soc. Neurosci. Abstr. 16, 1299.

Jones, D.L., G.J. Mogenson and M. Wu, 1981, Injections of dopaminergic, cholinergic, serotoninergic and gabaergic drugs into the nucleus accumbens: effects on locomotor activity in the rat, Neuropharmacology 20, 29.

Kennett, G.A. and T.P. Blackburn, 1991, Inhibition of agonist-induced dopamine release in the rat nucleus accumbens by BRL 46470, an in vivo dialysis study, Abstracts, 5-Hydroxytryptamine-CNS Receptors and Brain Function, Birmingham, UK, p. 103.

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

Laporte, A.M., E.J. Kidd, D. Vergé, H. Gozlan and M. Hamon, 1992, Autoradiographic mapping of central 5-HT_3 receptors, in: Central and Peripheral 5-HT_3 Receptors, ed. M. Hamon (Academic Press, London) p. 157.

Layer, R.T., N.J. Uretsky and L.J. Wallace, 1992, Effect of serotonergic agonists in the nucleus accumbens on d-amphetamine-stimulated locomotion, Life Sci. 50, 813.

Makanjuola, R.O.A., R.C. Dow and G.W. Ashcroft, 1980, Behavioural responses to stereotactically controlled injections of monoamine neurotransmitters into the accumbens and caudate-putamen nuclei, Psychopharmacology 71, 227.

McBain, S.L., M. Dineen, M. Weller, S. Taylor, P. Cooksey, P. Williams and T. Corn, 1992, Ondansetron: a double blind placebo controlled study in acute schizophrenia, Schizophren. Res. 6, 112.

Meltzer, H.Y., 1991, Studies of ondansetron in schizophrenia, in: Biological Psychiatry, Vol. 2, Proceedings of 5th World Congress of Biological Psychiatry, ed. G. Racagni, N. Brunello and T. Fukuda (Elsevier, Amsterdam) p. 891.

Minabe, Y., C.R. Ashby, Jr., J.E. Schwartz and R.Y. Wang, 1991a, The 5-HT_3 receptor antagonists LY 277359 and granisetron potentiate the suppressant action of apomorphine on the basal firing rate of ventral tegmental dopamine cells, Eur. J. Pharmacol. 209, 143.

Minabe, Y., C.R. Ashby, Jr. and R.Y. Wang, 1991b, The effect of acute and chronic LY 277359, a selective 5-HT_3 receptor antagonist, on the number of spontaneously active midbrain dopamine neurons, Eur. J. Pharmacol. 209, 151.

Minabe, Y., C.R. Ashby, Jr. and R.Y. Wang, 1992, Effects produced by acute and chronic treatment with granisetron alone or in combination with haloperidol on midbrain dopamine neurons, Eur. Neuropsychopharmacol. 2, 127.

Newcomer, J.W., W.O. Faustman, R.B. Zipursky and J.G. Csernansky, 1992, Zacopride in schizophrenia: a single-blind serotonin type 3 antagonist trial, Arch. Gen. Psychiat. 49, 751.

Parsons, L.H. and J.B. Justice, Jr., 1993, Perfusate serotonin increases extracellular dopamine in the nucleus accumbens as measured by in vivo microdialysis, Brain Res. 606, 195.

Paxinos, G. and C. Watson, 1986, The Rat Brain in Stereotaxic Coordinates, 2nd edn. (Academic Press, Sydney).

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.

Pijnenburg, A.J.J., W.M.M. Honig and J.M. Van Rossum, 1975, Effects of antagonists upon locomotor stimulation induced by injection of dopamine and noradrenaline into the nucleus accumbens of nialamide-pretreated rats, Psychopharmacologia (Berlin) 41, 175.

Prisco, S., M. Pessia, A. Ceci, F. Borsini and E. Esposito, 1992, Chronic treatment with DAU 6215, a new 5-HT_3 receptor antagonist, causes a

selective decrease in the number of spontaneously active dopaminergic neurons in the rat ventral tegmental area, Eur. J. Pharmacol. 214, 13.

Rasmussen, K., M.E. Stockton and J.F. Czachura, 1991, The 5-HT_3 receptor antagonist zatosetron decreases the number of spontaneously active A10 dopamine neurons, Eur. J. Pharmacol. 205, 113.

Silverstone, P.H., B. Johnson and P.J. Cowen, 1992, Does ondansetron attenuate amphetamine-induced behaviour in human volunteers?, Psychopharmacology 107, 140.

Sorensen, S.M., T.M. Humphreys and M.G. Palfreyman, 1989, Effect of acute and chronic MDL 73,147EF, a 5-HT_3 receptor antagonist, on A9 and A10 dopamine neurons, Eur. J. Pharmacol. 163, 115.

Tricklebank, M.D, 1989, Interactions between dopamine and 5-HT_3 receptors suggest new treatments for psychosis and drug addiction, Trends Pharmacol. Sci. 10, 127.

Tricklebank, M.D., 1992, The functional importance of 5-HT_3 receptors in the interactions between serotoninergic and dopaminergic systems in the CNS, in: Central and Peripheral 5-HT_3 Receptors, ed. M. Hamon (Academic Press, London) p. 189.

Volonté, M., A. Ceci and F. Borsini, 1992, Effect of haloperidol and clozapine on (+)SKF 10,047-induced dopamine release: role of 5-HT_3 receptors, Eur. J. Pharmacol. 213, 163.

Wang, R.Y. and L.H. Jiang, 1990, Effects of the 5-HT_3 receptor agonist 2-methyl-5HT on the neuronal activity of A10 DA cells, Soc. Neurosci. Abstr. 16, 1299.

White, A., T.H. Corn, C. Feetham and C. Faulconbridge, 1991, Ondansetron in treatment of schizophrenia, Lancet 337, 1173.

Yoshimoto, K., W.J. McBride, L. Lumeng and T.K. Li, 1992, Alcohol stimulates the release of dopamine and serotonin in the nucleus accumbens, Alcohol 9, 17.

Zoldan, J., G. Friedberg, H. Goldberg-Stern and E. Melamed, 1993, Ondansetron for hallucinosis in advanced Parkinson's disease, Lancet 341, 562.