Br. J. Pharmacol. (1990), **99**, 227–232

Effect of 5-HT_3 receptor antagonists on responses to selective activation of mesolimbic dopaminergic pathways in the rat

[1]R.M. Hagan, B.J. Jones, C.C. Jordan & M.B. Tyers

Department of Neuropharmacology, Glaxo Group Research Ltd., Ware, Hertfordshire, SG12 0DP

1 The effects of 5-hydroxytryptamine$_3$ (5-HT_3) receptor antagonists on the behavioural hyperactivity response which results from injection of the neurokinin receptor agonist [pGlu^5, MePhe^8, Sar^9]-substance P (5–11) (DiMe-C7) into the ventral tegmental area (VTA) of the rat midbrain have been determined.

2 Subcutaneous administration of ondansetron (GR38032) (0.001–0.3 mg kg^{-1}), GR65630 (0.01 mg kg^{-1}), ICS 205–930 (0.1 mg kg^{-1}) and MDL 72222 (0.1 mg kg^{-1}), inhibited the DiMe-C7-induced hyperactivity response.

3 The effects of ondansetron on DiMe-C7-induced changes in dopamine and 5-HT metabolism in discrete areas of rat forebrain were studied in order to investigate further the possible mechanism of action of 5-HT_3 antagonists in modifying mesolimbic dopaminergic systems.

4 Intra-VTA administration of DiMe-C7 increased levels of dihydroxyphenylacetic acid (DOPAC) in the nucleus accumbens, olfactory tubercules and right amygdala, indicating increased mesolimbic dopamine metabolism. DOPAC levels were not significantly increased in the frontal cortex, left amygdala or striatum. Dopamine levels were not altered in any of these brain areas. DiMe-C7 also increased 5-hydroxyindoleacetic acid (5-HIAA) levels in the amygdala but this was only statistically significant in the right amygdala. 5-HT levels were not changed significantly by DiMe-C7 treatment.

5 In control rats, pretreatment with ondansetron (0.1 mg kg^{-1}) had no effect on the levels of dopamine, 5-HT or their metabolites, but in rats given DiMe-C7, ondansetron significantly inhibited the increase in DOPAC levels in the nucleus accumbens.

6 These results are in agreement with the proposed facilitatory role of 5-HT_3 receptor activation on mesolimbic dopaminergic transmission, and suggest that 5-HT_3 antagonists may have important therapeutic indications for the treatment of CNS disorders in which mesolimbic dopamine systems are perturbed.

Introduction

The 5-hydroxytryptamine$_3$ (5-HT_3) receptor antagonists which include ondansetron (GR38032) (Butler *et al.*, 1988), ICS 205-930 (Richardson *et al.*, 1985), MDL 72222 (Fozard, 1984), zacopride (Smith *et al.*, 1988) and BRL43694 (Fake *et al.*, 1987) can reduce the behavioural consequences of raised mesolimbic dopamine activity in the rat and marmoset (Hagan *et al.*, 1987; Costall *et al.*, 1987a, b). The mechanism(s) underlying this action are unclear. Using radioligand binding with the 5-HT_3 receptor antagonist [^{3}H]-GR65630, Kilpatrick *et al.* (1987) have recently demonstrated that binding sites with the characteristics of 5-HT_3 receptors are present predominantly in limbic and cortical areas of the forebrain of the rat such as the entorhinal cortex, frontal cortex, amygdala and nucleus accumbens/olfactory tubercules. In contrast, few binding sites are found in striatum and lower brain areas, with the exception of the area postrema, where the highest density of 5-HT_3 receptors exist. 5-HT_3 receptors in the area postrema may mediate the anti-emetic properties of 5-HT_3 antagonists in other species (Higgins *et al.*, 1989).

It is possible, therefore, that the effect of 5-HT_3 receptor antagonists on dopamine-induced hyperactivity responses may be mediated through limbic or cortical 5-HT_3 receptors. Indeed, Costall *et al.* (1987a,b) have shown that 5-HT_3 receptor antagonists inhibit raised mesolimbic dopaminergic activity through an action on limbic 5-HT_3 receptors. Since 5-HT_3 receptor antagonists have no overt effect on normal rat behaviour, it appears necessary to excite mesolimbic dopaminergic pathways to demonstrate their behavioural effects.

We have previously shown (Hagan *et al.*, 1987) the effects of ondansetron on mesolimbic dopaminergic systems, utilizing a model of dopamine-mediated behavioural hyperactivity in the rat, first described by Eison *et al.* (1982a). In this model, the injection of the metabolically stable neurokinin receptor agonist, [pGlu^5, MePhe^8, Sar^9]-substance P(5–11) (DiMe-C7), into the ventral tegmental area (VTA) causes a complex behavioural hyperactivity response which is thought to arise, at least in part, from activation of the mesolimbic dopaminergic pathway. The increased locomotor activity induced by DiMe-C7 can be blocked by haloperidol (Eison *et al.*, 1982b) and is accompanied by increases in dopamine metabolism in the mesolimbic and mesocortical areas of the forebrain (Elliott *et al.*, 1986). We found that ondansetron reduced both the hyperactivity response and the increase in dopamine metabolism produced by the DiMe-C7 treatment in the nucleus accumbens. We have now extended these observations to include other 5-HT_3 receptor antagonists and have further investigated the effects of ondansetron on DiMe-C7-induced changes in dopamine metabolism in other areas of rat forebrain, including the amygdala. By use of discrete injection techniques, other workers have shown that some of the behavioural effects of 5-HT_3 receptor antagonists may be mediated by the amygdala (see Costall *et al.*, 1987a, 1989). Interestingly, ondansetron was found to be more effective at inhibiting the hyperactivity response to unilateral infusion of dopamine into the left amygdala when it was injected into the non-infused right amygdala (Costall *et al.*, 1987a). Therefore, in the present experiments, possible neurochemical effects were investigated in both left and right amygdala. In order to investigate the involvement of 5-HT neurones in the hyperactivity response we have also looked at 5-HT metabolism in DiMe-C7-treated animals.

Methods

Animals

Male Random-Hooded strain rats (Rodent Breeding Unit, Glaxo Group Research, Ware) weighing 250–320 g were used.

[1] Author for correspondence.

The rats were housed in groups of 5 at a temperature of 20°C on a 12 h light-dark cycle of lights on between 06 h 30 min and 18 h 30 min. Rats were fed Rat and Mouse No. 1 expanded maintenance diet (Special Diet Services) and allowed water *ad libitum*.

Stereotaxic surgery

Rats were anaesthetized with chloral hydrate (550 mg kg^{-1} s.c.) and placed in a Kopf stereotaxic frame. Guide cannulae, consisting of stainless steel tubing (23 gauge) in a perspex holder, were implanted bilaterally, positioned stereotaxically 3.0 mm above the VTA (Ant. +3.0, Vert. −2.5, Lat. ±1.0) with the incisor bar 5.0 mm above the inter-aural line. Stainless steel stylets (30 gauge) were used to keep the guide cannulae patent during the two week recovery period. These extended 0.5 mm beyond the guide tips.

Intracerebral injections

Ten days after surgery the stylets were removed and replaced, to facilitate intracerebral injections. Four days later, the rats were briefly restrained, their stylets removed and intracerebral injection needles (30 gauge) inserted bilaterally into the guide cannulae such that they terminated 3.0 mm below the guide tips. Peptide solutions or vehicle were infused manually over a 30 s period with a micrometer screw gauge, calibrated to deliver a volume of 1 μl by means of Bolab V3 polythene tubing attached to an Agla glass syringe. The injection needles were left in place for a further 60 s, then removed and the stylets replaced. Rats were used on a maximum of two separate occasions, with a 7 day period between testing.

Behavioural testing

Testing was conducted between 08 h 00 min and 14 h 00 min. Rats were allowed to adapt to the test room for at least 1 h before experimentation began. In experiments where rats were to be pretreated with the drug under test or vehicle, this was given subcutaneously into the back of the neck and the animals were then placed immediately into individual perspex observation boxes (25 × 20 × 20 cm high). Locomotor activity was assessed by means of a single infra-red beam photocell-unit placed along the longer axis of the box and located off-centre 4 cm above the floor of the box and 8 cm from the side. Infra-red beam crossings were counted by a microcomputer in 10 min epochs. After 40 min, the rats were removed from the boxes whilst intra-cerebral injections were made and then immediately replaced in the same box for a further period of locomotor counting (typically 90 min). In some experiments, wet dog shakes and rearing were also counted by an observer. In experiments designed to show the neurochemical changes produced by the treatments, the above procedures were adhered to, except that 50 min after intra-cerebral injection the rats were removed from the boxes and taken into a separate room.

Neurochemical experiments

Following removal from the observation room the rats were immediately killed, their brains rapidly removed and several brain areas dissected out from coronal sections with the aid of a stainless-steel punch. With the exception of the amygdala, tissues taken from the left and right hemisphere were pooled. These tissues were then immediately homogenised in 250 μl 0.1 M perchloric acid (containing 0.1 mM disodium EDTA, 0.26 mM sodium metabisulphite and 50 ng ml^{-1} dihydroxybenzylamine (DHBA)) by means of an ultrasonic probe (MSE Soniprep). Homogenised samples were then frozen in liquid nitrogen until centrifugation. Homogenates were centrifuged at 4°C for 3 min at 18,000 r.p.m. in a Burkard Coolspin bench centrifuge. The resulting supernatants were frozen in liquid nitrogen and stored at −70°C until analysed. The pellets were resuspended in 0.1 M NaOH and protein content was measured by the method of Lowry *et al.* (1951).

High performance liquid chromatography (h.p.l.c.) with electrochemical detection

Baseline separation of dopamine, dihydroxyphenylacetic acid (DOPAC), 5-HT, 5-hydroxyindoleacetic acid (5-HIAA) and DHBA in standard mixtures and the supernatants was accomplished by h.p.l.c. separation on a Waters Nova-Pak C_{18} reverse-phase radial compression column (mobile phase: 0.1 M disodium hydrogen phosphate buffer, pH 2.85, containing 4 mM heptane sulphonic acid, 0.2 mM disodium EDTA, 9% v/v acetonitrile and 3.5% v/v methanol, flow rate 1.5 ml min^{-1}). The eluate was analysed by oxidative electrochemical detection with an ESA model 5100A detector with model 5011 electrodes, the first set at +0.1 V to act as a filter and the second set at +0.45 V for chromatographic peak detection. Detected peaks were analysed by a Trivector Trio computing integrator, which calculated the area under peaks of interest. The amounts of dopamine, DOPAC, 5-HT and 5-HIAA appearing in the samples were quantitated with reference to external standard calibration curves for each amine and metabolite (5–250 pg, repeated every 24 samples) with correction for the known amount of the internal standard DHBA which was present in each sample. The minimum detectable level of the amines and their metabolites which could be reliably quantitated by this procedure was 5 pg. Tissue levels were expressed as ng mg^{-1} of tissue protein.

Histological examination

Following the described experimental procedures all animals were killed and their brains removed. Sections of brain containing the VTA were preserved in buffered formalin solution for subsequent histological examination by section with a freezing microtome. The injection sites were located from the tracks of the injection needles. Rats in which cannulae were sited outside the VTA were excluded from the study.

Statistical analyses

Results were analysed by analysis of variance followed by Student's *t* test or Dunnett's *t* test for multiple comparisons.

Drugs and solutions

All reagents used were of analytical grade (Analar, BDH). Methanol and acetonitrile for h.p.l.c. were of h.p.l.c. grade (Rathburn). Heptane sulphonic acid was obtained from Kodak. Except where indicated, pure (h.p.l.c. grade) water was used throughout.

DiMe-C7 (Bachem (U.K.) Ltd.) was dissolved to a final concentration of 10 mM in nitrogen de-gassed water, which had been filtered through a 0.2 μm filter (Millipore). Aliquots of this solution were stored at −70°C until required. A sample of each aliquoted batch of DiMe-C7 was subjected to analysis for amino acid composition and peptide content by the Medicinal Chemistry Department of Glaxo Group Research, Greenford. Correction was then made for peptide content in the aliquots.

Ondansetron (1,2,3,9-tetrahydro-9-methyl-3-[(2-methyl-1*H*-imidazol-1-yl)-methyl]-4*H*-carbazol-4-one); GR65630 (3-(5-methyl-1*H*-imidazol-4-yl)-1-(1-methyl-1*H*-indol-3-yl)-1-propanone) and MDL 72222 (1αH,3α,5αH-tropan-3-yl-3,5-dichlorobenzoate) were all synthesised in the Department of Chemical Research, Glaxo Group Research, Ware). ICS 205 930 ((3α-tropanyl)-1*H*-indole-3-carboxylic acid ester) was a gift from Dr G. Engel, Sandoz, Basle.

All drugs for subcutaneous injection were dissolved in sterile water for injection and diluted in sterile 0.9% w/v saline solution. Drug solutions were injected as base equivalents in dose volumes of 0.1 ml per 100 g body weight.

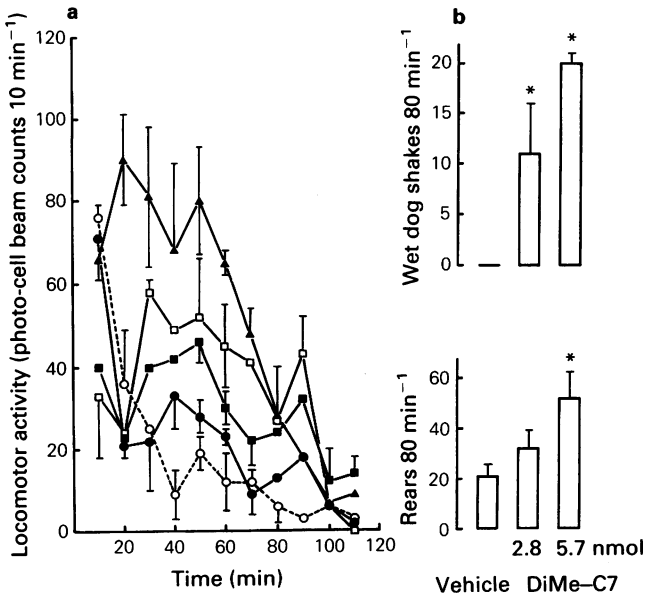

Figure 1 Responses to bilateral injection of DiMe-C7 into the ventral tegmental area (VTA). (a) Locomotor activity response (in counts 10 min^{-1}) to injection of vehicle (○) or DiMe-C7 (●) 1.4, (□) 2.8, (□) 5.7 and (△) 11.5 nmol intra-VTA. Each point represents the mean of 4–6 animals; vertical lines show s.e.mean. (b) Wet dog shake and rearing responses, expressed as total number observed in the 80 min period following intra-VTA injection for the doses of DiMe-C7 indicated. Each column represents the mean of 4–6 animals; vertical bars show s.e.mean. Statistically significant differences from vehicle-treated control animals are shown. * $P < 0.05$.

Standard compounds for the h.p.l.c. analyses were obtained from Sigma. These were dissolved to a final concentration of 50 ng ml^{-1} of base in 0.1 M perchloric acid (containing 0.1 mM disodium EDTA and 0.26 mM sodium metabisulphite).

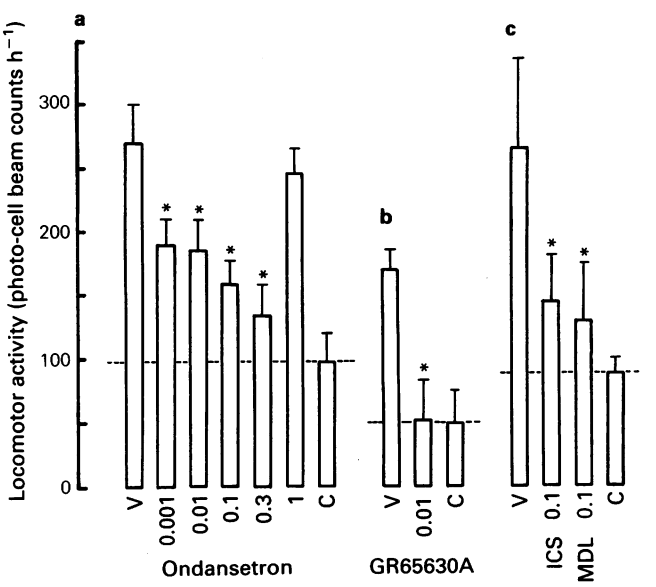

Figure 2 Effect of subcutaneous administration of (a) ondansetron, (b) GR65630A, (c) ICS 205 930 and MDL 72222 on the locomotor hyperactivity induced by injection of DiMe-C7 into the ventral tegmental area (VTA). The response of those animals receiving subcutaneous vehicle pretreatment before DiMe-C7 are indicated as V, those receiving subcutaneous vehicle followed by vehicle into the VTA as C. Pretreatments were administered 40 min before intracerebral injections. In each case the dose of DiMe-C7 was 2.8 nmol, except in the experiments with ondansetron, where a dose of 5.7 nmol was used. Each column shows the mean for 4–12 animals; vertical bars show s.e.mean. By use of Dunnett's t test, the statistically significant differences from animals receiving vehicle followed by DiMe-C7 are shown. * $P < 0.05$.

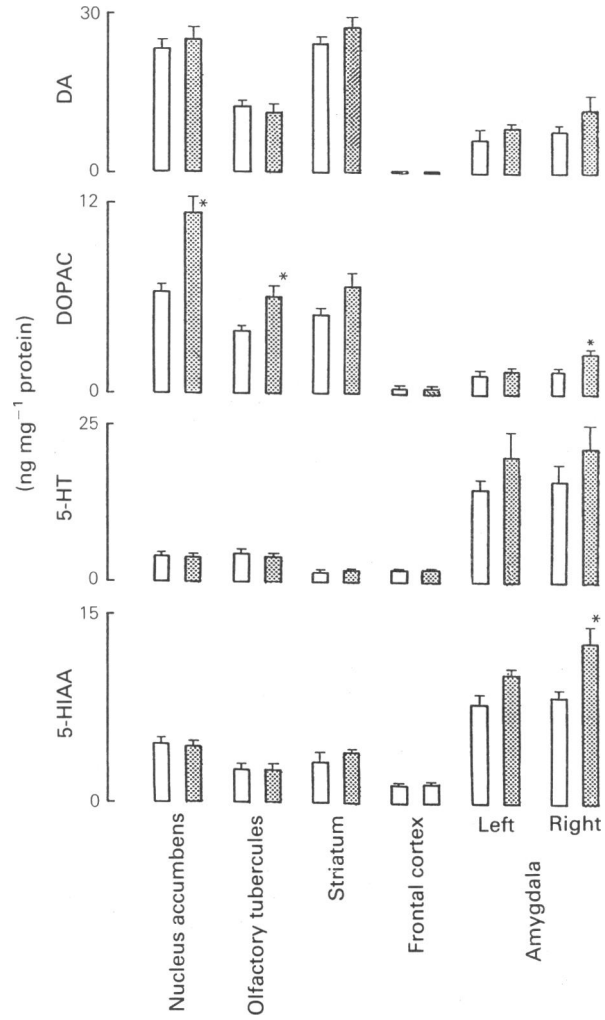

Figure 3 Effect of DiMe-C7 (5.7 nmol, injected into the ventral tegmental area (VTA), stippled columns) on the levels of dopamine (DA), dihydroxyphenylacetic acid (DOPAC), 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in the nucleus accumbens, olfactory tubercules, striatum, frontal cortex, left and right amygdala. Each column shows the mean for 4–8 animals; vertical bars indicate s.e.mean. Statistically significant differences from animals receiving vehicle intra-VTA are shown. * $P < 0.05$.

Results

Effect of intra-VTA DiMe-C7 on locomotor activity

Bilateral intra-VTA injection of DiMe-C7 (1.4–11.4 nmol) caused a behavioural hyperactivity syndrome which included increased locomotor activity, increased rearing and wet-dog shakes (Figure 1). Both the time-course and magnitude of the locomotor hyperactivity response were dose-dependent. At the lower doses of DiMe-C7 (1.4, 2.8 and 5.7 nmol) the increase in locomotor activity in some animals was preceded by a period of reduced locomotor activity with respect to vehicle controls. Marked lachrimation was often noted during this period. At these doses the peak hyperactivity response occurred at 30–50 min. In contrast, at the highest dose of DiMe-C7 (11.4 nmol) the onset of the hyperactivity was more immediate and the peak response occurred after 20 min. However, there was also a second peak of hyperactivity which occurred after 50 min. At all doses of DiMe-C7, locomotor activity returned to control levels by 100 min.

In further studies where the rats received subcutaneous pretreatments, doses of DiMe-C7 were used which produced a submaximal hyperactivity. In these experiments, the rats were

acclimatized to the test environment and the initial hypoactivity when compared to control animals was reduced or absent. The locomotor hyperactivity response of the rats was expressed as the mean total counts (± s.e.mean) in the period 20–80 min after intra-VTA injection.

Effect of 5-HT$_3$ antagonists on the locomotor hyperactivity-induced by DiMe-C7

Pretreatment (40 min s.c.) with ondansetron (0.001–0.3 mg kg^{-1}), GR65630A (0.01 mg kg^{-1}), ICS 205 930 (0.1 mg kg^{-1}) or MDL 72222 (0.1 mg kg^{-1}) inhibited, in a statistically significant manner, the hyperactivity response to DiMe-C7 (2.8 or 5.7 nmol) (Figure 2). A high dose of ondansetron (1 mg kg^{-1}), was without effect on the hyperactivity response. None of these compounds reduced spontaneous locomotion in the rats, measured in the 30 min period prior to DiMe-C7 injection and wet dog shakes induced by DiMe-C7 were not affected by pretreatment with ondansetron (data not shown).

Effect of ondansetron on DiMe-C7-induced changes in dopamine and 5-HT metabolism

Intra-VTA injection of DiMe-C7 (5.7 nmol) increased, in a statistically significant manner ($P < 0.05$), the levels of DOPAC in the nucleus accumbens (+59%), olfactory tubercules (+41%) and right amygdala (+78%) but not the left amygdala (+25%), frontal cortex (+2%) or striatum (+31%) (Figure 3). DiMe-C7 had no significant effect ($P < 0.05$) on the levels of dopamine in any of these brain areas. DiMe-C7 increased 5-HIAA levels in the amygdala but these changes only reached statistical significance in the right amygdala, where 5-HIAA levels were increased by 54% ($P < 0.05$, Figure 3). 5-HT levels were also increased in the amygdala, but these changes were not significant in either hemisphere ($P > 0.05$).

In control rats, pretreatment with ondansetron (0.1 mg kg^{-1}) had no significant effect ($P > 0.05$) on DOPAC or 5-HIAA levels, but in rats treated with DiMe-C7, ondansetron inhibited the increase in DOPAC levels in the nucleus accumbens and right amygdala (Figure 4), but this effect was only significant in the nucleus accumbens ($P < 0.05$). The increase in 5-HIAA levels in the right amygdala also appeared to be reduced by ondansetron, but this reduction was not significant ($P > 0.05$) when compared to animals treated with DiMe-C7 and was still significantly different ($P < 0.05$) from animals receiving ondansetron alone.

Histology

Injection sites were confirmed by subsequent histological examination. In general, injection needle tracks were found to terminate within antero-medial aspects of the VTA. Data from those animals with injection tracks terminating within the VTA (approximately 80% of animals tested) were included in the analyses of results. Representative data from 25 animals with injection tracks terminating in the VTA are shown in Figure 5.

Discussion

Administration of certain neurokinin agonists into the VTA of rat brain enhances locomotor activity (Elliott & Iversen, 1986). This effect can be produced by substance P (Stinus *et al.*, 1978), but is short-lived. Administration into the VTA of the metabolically more stable analogue of substance P, DiMe-C7, mimics the locomotor stimulant effects of substance P, but the duration of effect is much increased (Eison *et al.* 1982a). In the present experiments, intra-VTA administration of DiMe-C7 produced a dose-related, complex behavioural hyperactivity syndrome, which included increased locomotion, rearing and wet dog shakes. These effects are similar to those described by Eison *et al.* (1982b), although the time course of

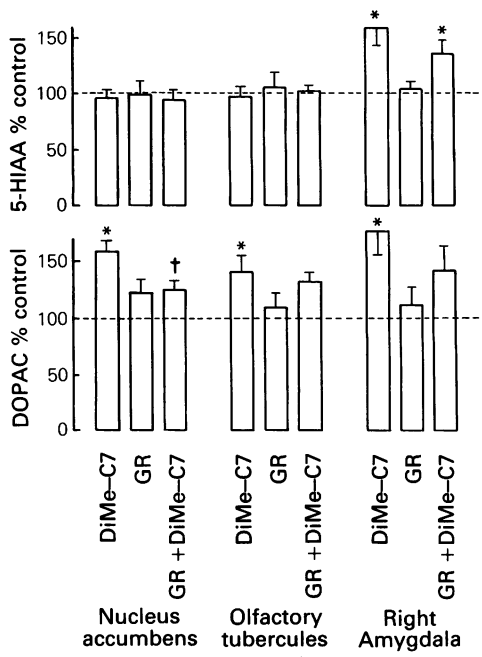

Figure 4 Effect of ondansetron (0.1 mg kg^{-1}, GR) on the changes in dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5-HIAA), induced by an injection of DiMe-C7 (5.7 nmol) into the ventral tegmental area (VTA), in the nucleus accumbens, olfactory tubercules and right amygdala. Columns represent the mean percentage of the metabolite level in control animals. Vertical bars indicate s.e.mean. Statistically significant differences are shown. * $P < 0.05$ versus vehicle controls; † $P < 0.05$ versus DiMe-C7-treated animals.

the locomotor hyperactivity which we observed was somewhat different, as described in detail in the results section.

The reason for this difference in profile between the present results and those of Eison *et al.* (1982b) is unclear, but may reflect rat strain differences or small differences in cannula placement. We are currently investigating the possibility that the relative lack of selectivity of DiMe-C7 between neurokinin receptors may also be important. DiMe-C7 has agonist activity at NK_1 and NK_3 receptors in peripheral tissues *in vitro* (Regoli *et al.*, 1987). It is therefore possible that a combination of actions at more than one neurokinin receptor subtype in

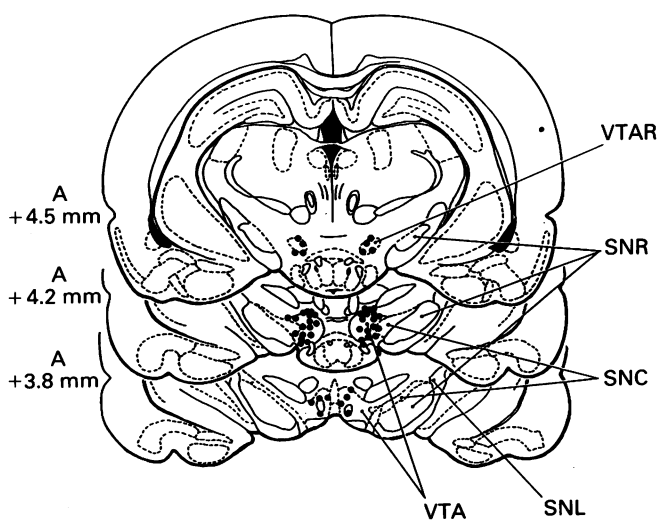

Figure 5 Diagrammatic representation of the sites of DiMe-C7 injection (●). Representative data from 25 animals with injection tracks terminating in the ventral tegmental area (VTA) are shown. The coordinates are according to Paxinos & Watson (1986). SNR, substantial nigra, pars compacta; SNL, substantia nigra, pars lateralis; SNR, substantia nigra, pars reticulata; VTA, ventral tegmental area; VTAR, ventral tegmental area pars rostralis.

the VTA may mediate the effects of DiMe-C7. The relative contribution from each action may determine the overall activity profile. For example, the ability of DiMe-C7 to produce wet dog shakes after central administration to rats is not mimicked by substance P (Eison *et al.*, 1982b) but is mimicked by the selective NK_3 agonist senktide (Stoessl *et al.*, 1988). This effect of senktide presumably involves activation of 5-hydroxytryptaminergic mechanisms since it can be blocked by the selective 5-HT$_2$ receptor antagonist ketanserin. In our experiments, ondansetron was found to have no effect on DiMe-C7-induced wet dog shakes. This is in agreement with the lack of effect of low doses of the 5-HT$_3$ receptor antagonist ICS 205-930 on wet dog shakes induced by the 5-HT precursor, 5-hydroxytryptophan (Shearman & Toksvai, 1987).

The hyperactivity-induced by DiMe-C7 is almost completely abolished by a low dose of haloperidol (Hagan *et al.*, 1987; Eison *et al.*, 1982b), supporting the hypothesis that activation of dopamine systems is involved in the hyperactivity induced by the intra-VTA injection of DiMe-C7. In the present study, we have demonstrated that the 5-HT$_3$ antagonists ondansetron, GR65630 (Kilpatrick *et al.*, 1987), ICS 205-930 and MDL 72222 also reduce this hyperactivity response. This effect would appear to be independent of any effect of the 5-HT$_3$ receptor antagonists on spontaneous locomotor activity, which was not affected in the 30 min period before DiMe-C7 administration. It could be argued that an intrinsic effect on locomotor activity with a longer latency could account for the observed effects. Whilst we cannot rule out this possibility, it seems unlikely, since we have shown that, at a dose (0.1 mg kg^{-1}) which was effective in the present study, ondansetron has no effect on spontaneous locomotion in rats over an 8 h period (unpublished observations). Our results support previous observations (Costall *et al.*, 1987a; Hagan *et al.*, 1987) and suggest that activation of 5-HT$_3$ receptors may facilitate overactivity in mesolimbic dopaminergic systems. Although ondansetron was active at doses as low as 1 μg kg^{-1}, its effect was not maintained at the highest dose of antagonist tested (1 mg kg^{-1}). This decline in effect at supra-effective doses has also been demonstrated for several 5-HT$_3$ receptor antagonists in the continuous dopamine infusion model of hyperactivity in the rat (Costall *et al.*, 1987b). However, it should be noted that at 1 mg kg^{-1} ondansetron was effective at inhibiting intra-accumbens amphetamine-induced hyperactivity (Costall *et al.*, 1987a). In our experiments, the lack of effect of the highest dose of ondansetron was not accompanied by any overt changes in behaviour in the rats and spontaneous locomotor activity was not affected. The mechanism underlying this loss of effect is unknown but may indicate a secondary opposing action of 5-HT$_3$ receptor antagonists which is only observed at high doses.

In order to further our understanding of the mechanisms by which 5-HT$_3$ antagonists reduce mesolimbic hyperactivity, we investigated the effect of ondansetron on DiMe-C7-induced changes in dopamine and 5-HT metabolism in several areas of rat forebrain. DiMe-C7 significantly increased dopamine metabolism in the nucleus accumbens and olfactory tubercules. In the amygdala, DOPAC and 5-HIAA levels were increased in both hemispheres, but these changes were more pronounced in the right amygdala, where both DOPAC and 5-HIAA levels were significantly increased. In control animals, there was no significant difference between left and right amygdala in the levels of either metabolite. This apparent asymmetry in the effect of bilateral injections of DiMe-C7 is puzzling. One possibility is that there is laterality in the response to DiMe-C7. Alternatively, these changes could arise out of small differences in cannula placement between the left and right VTA. The levels of dopamine and DOPAC in the frontal cortex supernatants were low, approximately 3–5 times the reliable detection limits of the h.p.l.c. with electrochemical detection assay. Hence, the data must be interpreted with some caution. However, the complete lack of effect of DiMe-C7 on frontal cortex dopamine metabolism is surprising, given that this structure is innervated by dopaminergic A10 neurones and that Elliott *et al.* (1986) found that a similar treatment with DiMe-C7 caused marked increases (+120%) in DOPAC levels in pre-frontal cortex. Possibly, the dopaminergic neurones which project to the frontal cortex may not respond in the same way to DiMe-C7 as those projecting to the pre-frontal cortex. The localization of the major component of the DiMe-C7 response to mesolimbic dopaminergic systems is supported by the observation that DiMe-C7 had a smaller, not statistically significant effect on striatal dopamine metabolism (31% increase in DOPAC levels, compared to 59% in the nucleus accumbens). DiMe-C7 also produces locomotor hyperactivity after injection into the substantia nigra (SN) of rats (Eison *et al.*, 1982b). Thus, the smaller effect of intra-VTA injection of DiMe-C7 on striatal DOPAC levels suggests that the contribution of striatal dopaminergic neurones to the locomotor hyperactivity induced by intra-VTA DiMe-C7 in the behavioural experiments is not major; although, it cannot be ruled out, given the likely spread by diffusion of the injected peptide to the substantia nigra (see Eison *et al.*, 1982a) and the existence of a population of VTA neurones which project to the ventral striatum (Fallon & Moore, 1978).

In common with its lack of effect on overt normal rat behaviour, ondansetron had no effect on either dopamine or 5-HT metabolism in control animals, supporting previous observations that it has no effect on normal levels of activity of dopamine and 5-HT neurones in cortical and limbic brain areas (Hagan *et al.*, 1987; 1988). In contrast, when mesolimbic dopaminergic activity was stimulated by DiMe-C7, ondansetron reduced both the resulting behavioural hyperactivity and the increase in nucleus accumbens DOPAC levels. Ondansetron has no significant antagonist activity at neurokinin receptors *in vivo* (unpublished data), nor does it inhibit neuronal dopamine uptake (A. Bradbury, personal communication) or monoamine oxidase A or B *in vitro* (M. Dixon, personal communication). Hence, the present results may indicate that ondansetron antagonises the peptide-induced release of mesolimbic dopamine by an action on 5-HT$_3$ receptors. This would imply that DiMe-C7 can increase the release of 5-HT, which then acts on 5-HT$_3$ receptors to amplify the mesolimbic dopaminergic activation. Indeed, the present experiments show that DiMe-C7 increases 5-HIAA levels in the amygdala, suggesting that 5-HT release may be increased here and, thus, that this region may be involved in the observed effects. In support of this hypothesis, it is known that the amygdala contains significant numbers of 5-HT$_3$ receptor binding sites (Kilpatrick *et al.*, 1987). Furthermore, administration of ondansetron into the central nucleus of either left or right amygdala inhibits the hyperactivity response induced by dopamine infusion into the left amygdala (Costall *et al.*, 1987a). Ondansetron had no statistically significant effect on DiMe-C7-induced increase in 5-HIAA levels in the amygdala, suggesting that it does not work by reducing the release of 5-HT. To date, no direct evidence exists that 5-HT, acting at 5-HT$_3$ receptors in the amygdala can facilitate mesolimbic dopamine release. However, Blandina *et al.* (1988) have recently shown that the 5-HT$_3$ receptor antagonist ICS 205–930 can reduce the 5-HT-induced increase in spontaneous overflow of endogenous dopamine from superfused slices of rat brain striatum, supporting the concept that 5-HT$_3$ receptor antagonists can modulate dopamine release. Further studies are required to demonstrate whether this effect can be observed in limbic areas, in particular, the amygdala.

These results may have implications for the use of 5-HT$_3$ receptor antagonists in man. Circumstantial evidence suggests that a disturbance of mesolimbic dopaminergic activity may underlie the symptoms of acute psychoses. Thus, by controlling the effects of excessive dopamine, 5-HT$_3$ receptor antagonists may be useful in the treatment of schizophrenia. In addition, activation of the VTA, leading to enhanced mesocortical and mesolimbic dopaminergic activity is thought to be important in the rewarding action of a number of drugs of abuse, including narcotic analgesics, alcohol, nicotine and

cocaine (see Wise & Bozarth, 1984; Bozarth & Wise, 1986). Therefore, 5-HT_3 receptor antagonists may be of use in the treatment of drug dependence and to alleviate the craving which occurs during drug withdrawal. Recent evidence suggests that ondansetron attenuates the symptoms of withdrawal from chronic alcohol intake in marmosets (Oakley *et al.*, 1988; Costall *et al.*, 1988) and inhibits the decrease in social interaction seen in rats following withdrawal from chronic diazepam treatment given peripherally or directly into the amygdala (Costall *et al.*, 1989). Moreover, ICS 205-930 and MDL 72222 can reduce place preference in rats induced by morphine or nicotine (Carboni *et al.*, 1989).

We would like to thank Lorraine Fallaize, Lyndsey Morgan and Jane Burridge for their excellent technical assistance, and Tessa Parker for typing this manuscript.

References

BOZARTH, M.A. & WISE, R.A. (1986). Involvement of the ventral tegmental dopamine system in opioid and psychomotor stimulant reinforcement. *Natl. Inst. Drug. Abuse Res. Monogr. Ser.*, **67**, 190–196.

BLANDINA, P., GOLDFARB, J. & GREEN, J.P. (1988). Activation of a 5-HT_3 receptor releases dopamine from rat striatal slice. *Eur. J. Pharmacol.*, **155**, 349–350.

BUTLER, A., HILL, J.M., IRELAND, S.J., JORDAN, C.C. & TYERS, M.B. (1988). Pharmacological properties of GR38032F, a novel antagonist at 5-HT_3 receptors. *Br. J. Pharmacol.*, **94**, 397–412.

CARBONI, E., ACQUAS, E., LEONE, P. & DI CHIARA, G. (1989). 5-HT_3 receptor antagonists block morphine- and nicotine-but not amphetamine-induced reward. *Psychopharmacology*, **97**, 175–178.

COSTALL, B., DOMENEY, A.M. & NAYLOR, R.J. (1982). Behavioural and biochemical consequences of persistent over-stimulation of mesolimbic dopamine systems in the rat. *Neuropharmacol.*, **24**, 327–335.

COSTALL, B., DOMENEY, A.M., NAYLOR, R.J. & TYERS, M.B. (1987a). 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**, 881–894.

COSTALL, B., DOMENEY, A.M., NAYLOR, R.J. & TYERS, M.B. (1987b). Inhibition by 5-HT_3 antagonists of hyperactivity caused by dopamine infusion into the rat accumbens. *Br. J. Pharmacol.*, **93**, 194P.

COSTALL, B., DOMENEY, A.M., JONES, B.J., KELLY, M.E., GERRARD, P.A., NAYLOR, R.J. & TYERS, M.B. (1988). Influence of GR38032F on the behavioural consequences of ceasing sub-chronic treatment with drugs of abuse. *Br. J. Pharmacol.*, **95**, 905P.

COSTALL, B., DOMENEY, A.M., GERRARD, P.A., KELLY, M.E., NAYLOR, R.J. & TYERS, M.B. (1989). Inhibition by ondansetron of behavioural effects in the rat which follow withdrawal from treatment with drugs of abuse. *Br. J. Pharmacol.*, **96**, 340P.

EISON, A.S., EISON, M.S. & IVERSEN, S.D. (1982b). The behavioural effects of a novel substance P analogue following infusion into the ventral tegmental area or substantia nigra of the rat brain. *Brain Res.*, **238**, 137–152.

EISON, A.S., IVERSEN, S.D., SANDBERG, B.E.B., WATSON, S.P., HANLEY, M.R. & IVERSEN, L.L. (1982a). Substance P analog, DiMe-C7: evidence for stability in rat brain and prolonged central actions. *Science*, **215**, 188–190.

ELLIOTT, P.J., ALPERT, J.E., BANNON, M.J. & IVERSEN, S.D. (1986). Selective activation of mesolimbic and mesocortical dopamine metabolism in rat brain by infusion of a stable substance P analogue into the ventral tegmental area. *Brain Res.*, **363**, 145–147.

ELLIOTT, P.J. & IVERSEN, S.D. (1986). Behavioural effects of tachykinins and related peptides. *Brain Res.*, **381**, 68–76.

FAKE, C.S., KING, F.D. & SANGER, G.J. (1987). BRL43694: A potent and novel 5-HT_3 receptor antagonist. *Br. J. Pharmacol.*, **91**, 335P.

FALLON, J.H. & MOORE, R.Y. (1978). Catecholamine innervation of the basal forebrain: IV. Topography of the dopamine projection to the basal forebrain and neostriatum. *J. Comp. Neurol.*, **180**, 545–580.

FOZARD, J.R. (1984). MDL 72222: A potent and highly selective antagonist at neuronal 5-hydroxytryptamine receptors. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **326**, 36–44.

HAGAN, R.M., BUTLER, A., HILL, J.M., JORDAN, C.C., IRELAND, S.J. & TYERS, M.B. (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**, 303–305.

HAGAN, R.M., JONES, B.J., JORDAN, C.C. & TYERS, M.B. (1988). Effects of the 5-HT_3 receptor antagonist, GR38032F, on the synthesis and metabolism of 5-HT and dopamine in rat forebrain. *Br. J. Pharmacol.*, **95**, 867P.

HIGGINS, G.A., KILPATRICK, G.J., BUNCE, K.T., JONES, B.J. & TYERS, M.B. (1989). 5-HT_3 receptor antagonists injected into the area postrema inhibit cisplatin-induced emesis in the ferret. *Br. J. Pharmacol.*, **97**, 247–255.

KILPATRICK, G.J., JONES, B.J. & TYERS, M.B. (1987). Identification and distribution of 5-HT_3 receptors in rat brain using radioligand binding. *Nature*, **330**, 746–748.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275.

OAKLEY, N.R., JONES, B.J., TYERS, M.B., COSTALL, B. & DOMENEY, A.M. (1988). The effect of GR38032F on alcohol consumption in the marmoset. *Br. J. Pharmacol.*, **95**, 870P.

PAXINOS, G. & WATSON, C. (1986). *The Rat Brain in Stereotaxic Coordinates*. 2nd edition. Sydney: Academic Press.

REGOLI, D., DRAPEAU, G., DION, S. & D'ORLÉANS-JUSTE, P. (1987). Receptors for neurokinins in peripheral organs. In *Substance P and Neurokinins*, ed. Henry, J.L., Couture, R., Cuello, A.C., Pelletier, G.I., Quirion, R.K. & Regoli, D., pp 99–107, New York: Springer-Verlag.

RICHARDSON, B.P., ENGEL, G., DONATSCH, P. & STADLER, P.A. (1985). Identification of serotonin M-receptor subtypes and their specific blockade by a new class of drugs. *Nature*, **316**, 126–131.

SHEARMAN, G.T. & TOLCSVAI, L. (1987). Effect of the selective 5-HT_3 receptor antagonists ICS 205-930 and MDL 72222 on 5-HTP-induced head shaking and behavioural symptoms induced by 5-methoxy-N,N dimethyltryptamine in rats: comparison with some other 5-HT receptor antagonists. *Psychopharmacology*, **92**, 520–523.

SMITH, W.W., SANCILIO, L.F., OWERA-ATEPO, J.B., NAYLOR, R.J. & LAMBERT, L. (1988). Zacopride, a potent 5-HT_3 antagonist. *J. Pharm. Pharmacol.*, **40**, 301–302.

STINUS, L., KELLEY, A.E. & IVERSEN, S.D. (1978). Increased spontaneous activity following substance P infusion into A10 dopaminergic area. *Nature*, **276**, 616–618.

STOESSL, A.J., DOURISH, C.T. & IVERSEN, S.D. (1988). The NK-3 tachykinin agonist senktide elicits 5-HT-mediated behaviour following central or peripheral administration in mice and rats. *Br. J. Pharmacol.*, **94**, 285–287.

WISE, R.A. & BOZARTH, M.A. (1984). Brain reward circuitry: four circuit elements "wired" in apparent series. *Brain Res. Bulletin*, **12**, 203–208.

(Received March 6, 1989
Revised October 2, 1989
Accepted October 5, 1989)