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Effects of the 5-HT_3 receptor antagonist, GR38032F, on raised dopaminergic activity in the mesolimbic system of the rat and marmoset brain

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1 The ability of the selective 5-HT_3 receptor antagonist GR38032F to reduce raised mesolimbic dopaminergic activity was studied in behavioural experiments in the rat and marmoset.

2 GR38032F injected into the nucleus accumbens (0.01–1 ng) or peripherally (0.01–1 mg kg^{-1} i.p.) inhibited the locomotor hyperactivity caused by the acute intra-accumbens injection of amphetamine (10 μg) in the rat. Similar treatments with sulpiride and fluphenazine also inhibited the amphetamine-induced hyperactivity.

3 The peripheral administration of GR38032F (0.001–0.1 mg kg^{-1} i.p., b.d.) during a 13 day period of dopamine infusion (25 μg 24 h^{-1}) into the nucleus accumbens of the rat reduced the dopamine-induced hyperactivity response to control (vehicle infused) levels. Locomotor activity remained at control levels after discontinuing the dopamine/GR38032F treatment regimen.

4 The hyperactivity caused by the infusion of dopamine into the rat nucleus accumbens was also inhibited by fluphenazine (0.01–0.05 mg kg^{-1} i.p., b.d.), but locomotor activity was suppressed to levels below control values and a rebound hyperactivity occurred after discontinuation of the dopamine/fluphenazine treatment regimen.

5 The discontinuation of a concomitant 13 day intra-accumbens infusion of dopamine with haloperidol, 0.01 mg kg^{-1} i.p. t.d.s., caused a rebound hyperactivity. This hyperactivity was suppressed by GR38032F (0.001–0.1 mg kg^{-1} i.p.).

6 The unilateral infusion of dopamine (25 μg 24 h^{-1}, 13 days) into the left amygdala of rats having right hemispheric dominance (as measured in a turn preference test) caused locomotor hyperactivity. Intraperitoneal administration of GR38032F (0.1–100 μg kg^{-1}) or fluphenazine (0.025–0.1 mg kg^{-1}), and the intra-amygdaloid injection of GR38032F (0.1–100 ng) or fluphenazine (25–500 pg), either into the infused or non-infused side, inhibited the dopamine-induced locomotor hyperactivity.

7 Marmosets receiving bilaterial infusions of dopamine (25 μg 24 h^{-1} for 13 days) into the nucleus accumbens also exhibited increased locomotor activity. GR38032F (0.1–1.0 μg kg^{-1} t.d.s.), reduced the hyperactivity to control levels with no rebound hyperactivity following the discontinuation of the dopamine/GR38032F treatment regimen. Fluphenazine (0.01–2.5 mg kg^{-1} i.p., t.d.s.) also inhibited the hyperactivity, but locomotor activity was reduced to values below control levels and a rebound hyperactivity followed the discontinuation of the dopamine/fluphenazine treatment.

8 It is concluded that the 5-HT_3 receptor antagonist GR38032F, and the neuroleptic agents fluphenazine, sulpiride and haloperidol, can reduce raised mesolimbic dopaminergic activity in the rat and marmoset. GR38032F is distinguished from the dopamine receptor antagonists by, firstly, its ability to return the hyperactivity response to control values, without excessive suppression of locomotion even on enhanced dosage regimes and, secondly, by the lack of rebound hyperactivity following abrupt discontinuation of its treatment.

Introduction

Considerable evidence supports a regulatory role for the 5-hydroxytryptamine (5-HT) innervation to the nucleus accumbens on dopaminergic mechanisms in the limbic forebrain. For example, 5-HT injected into the nucleus accumbens can inhibit the hyperactivity resulting from local injections of dopamine or

amphetamine into the same nucleus (Costall *et al.*, 1976a; Carter & Pycock, 1978; Pycock *et al.*, 1978; Jones *et al.*, 1981) or can reduce the circling response to amphetamine following lesion of the nigrostriatal pathway (Jackson *et al.*, 1975). Further evidence is derived from observations that destruction of 5-HT nerve terminals in the nucleus accumbens by 5,7-dihydroxytryptamine leads to increased spontaneous and drug-induced locomotor activity (Carter & Pycock, 1979; Lyness & Moore, 1981), and that lesions of the medial raphé nucleus, which disrupt the 5-HT input to forebrain structures, markedly enhance the hyperactivity caused by dopamine injected into the nucleus accumbens (Costall *et al.*, 1976a). However, reports on the consequences of 5-HT injection into the nucleus accumbens are varied, from ineffectiveness to either an inhibitory or facilitatory influence (Jackson *et al.*, 1975; Pijnenburg *et al.*, 1975; Costall *et al.*, 1979; Makanjuola *et al.*, 1980).

These results indicate a complex interaction between 5-HT and dopamine systems to control locomotor activity, and evidence can be put forward to support either an inhibitory or facilitatory action. The aims of the present study were to use a selective 5-HT_3 receptor antagonist, GR38032F (Brittain *et al.*, 1987), and a selective 5-HT_3 receptor agonist, 2-methyl-5-hydroxytryptamine (Richardson *et al.*, 1985), to investigate the possibility that manipulations at the 5-HT_3 receptor subtype may provide a novel route for moderating the hyperactivity resulting from excess dopaminergic activity in the nucleus accumbens and amygdala. Throughout the studies comparisons have been made with dopamine antagonist neuroleptic agents.

Preliminary results have been presented at the British Pharmacological Society (Costall *et al.*, 1987).

Methods

Animals

Male Sprague Dawley (CD) Bradford strain rats weighing 300 ± 25 g and male common marmosets (*Callithrix jacchus*) weighing 350–450 g were used. The rats were housed in groups of 5 at a temperature of 21 ± 2°C on a 12 h light-dark cycle of lights-on between 07 h 00 min and 20 h 00 min. Rats were fed CRM diet (Labsure) and allowed water *ad libitum*.

Marmosets were housed two per cage and allowed food (Mazuri primate diet, S.D.S. Ltd, Essex) and water *ad libitum*. Once daily marmosets were also given an assortment of fruit and, once weekly, all marmosets were given a vitamin supplement (Duphasol 13/6-2; Duphar Veterinary Ltd, Southampton) in fruit juice. Holding rooms were maintained at 25 ± 1°C at a humidity of 55%. Rooms were illuminated for 12 h with a 12 h dark cycle, with lights-on between 07 h 00 min and 19 h 00 min. Simulated dawn and twilight periods were achieved using a single 60 W bulb illuminated 0.5 h before and after the main lights came on and went off respectively. During the 12 h dark period a single 60 W red bulb was illuminated to avoid complete darkness.

Experiments in the rat

Stereotaxic techniques Rats were anaesthetized with chloral hydrate (400 mg kg^{-1} s.c.) and placed in a Kopf stereotaxic frame. Chronically indwelling guide cannulae (constructed of stainless steel tubing 0.65 mm diameter held bilaterally in Perspex holders) were implanted using standard stereotaxic techniques to terminate 3.5 mm above the centre of the nucleus accumbens (Ant. 9.4, Vert. 0.0, Lat. 1.6) or 5.0 mm above the central nucleus of the amygdala (Ant. 5.8, Vert. −1.8, Lat, ± 4.5) (atlas of De Groot, 1959). The guides were kept patent during a 14 day recovery period using stainless steel stylets, 0.3 mm diameter, which extended 0.5 mm beyond the guide tips.

Intracerebral injection technique Rats were manually restrained and the stylets removed. Intracerebral injection cannulae, 0.3 mm diameter, were inserted and drugs delivered in a volume of 0.5 µl over 5 s (a further 55 s was allowed for deposition) from Hamilton syringes attached via polythene tubing to the injection units. Animals were used on a single occasion only.

Intracerebral infusion technique Fourteen days after the implantation of guide cannulae animals were anaesthetized with fluothane for the subcutaneous implantation in the scapula region of two Alzet osmotic minipumps. Each pump was attached via 40–45 mm Bolab V3 polythene tubing to stainless steel injection units (0.3 mm diameter with a 0.65 mm diameter cuff) which were made to fit permanently into the previously implanted guides in place of the stylets, but terminated bilaterally at the centre of the nucleus accumbens. A single osmotic minipump was used for the unilateral infusion into the amygdala. The pumps had been previously filled with a dopamine solution or its solvent and the entire injection unit primed overnight at 37°C (see Costall *et al.*, 1983). The pumps and injection units were carefully designed such that their implantation did not cause any obvious disturbance to the free movement of the animals. The pumps delivered dopamine or its solvent at a constant rate of 0.48 µl h^{-1} from the time of implantation and, although the pumps were designed to deliver solution for 14 days, removal on day 13 precluded any 'fall-off' effect.

Behavioural studies Behavioural experiments were conducted between 07 h 30 min and 21 h 30 min in a quiet room maintained at 22 ± 2°C. Rats were taken from the holding room and allowed 1 h to adapt to the new environment. Locomotor activity was assessed in individual, screened Perspex cages (25 × 15 × 15 cm high) (banked in groups of 30) each fitted with one photocell unit along the longer axis 3.5 cm from the side; this position has been found to minimize spurious activity counts due to, for example, preening and head movements when the animal is stationary. Interruptions of the light beam were recorded every 5 min. At this time animals were also observed for the presence of any non-specific change in locomotor activity, e.g. sedation, prostration, stereotyped movements, that could interfere with the recording of locomotor activity.

Preselection of rats for behavioural experiments In the experiments involving the use of the dopamine infusion technique, animals were pre-selected as follows:

Unilateral infusion into the central nucleus of the amygdala Before surgery rats were initially categorized according to 'hemispheric dominance' (Barnes *et al.*, 1987a) by measuring turn preference in an open field. Rats were lifted from their holding cages using two hands placed firmly on either side of the body, held for 2 or 3 s and then released into an open field. The direction of immediate movement, right or left, was noted. If the immediate response of the animal was to turn left then the right hemisphere was defined as dominant, the left sub-dominant, the designation being reversed if the immediate response of the animal was to turn right. Turn preference was determined by two independent assessors on a minimum of 5 occasions; testing was carried out once daily and was shown, in most animals, to be a consistent phenomenon. Animals showing a consistent response and characterized as right hemispheric dominant were selected for subsequent study and received dopamine/vehicle infusion into the amygdala of the left hemisphere.

Bilaterial infusion into the nucleus accumbens Animals were preselected according to their responsiveness to the locomotor stimulant effects of the dopamine agonist (−)-N-n-propylnorapomorphine ((−)-NPA). Briefly, rats received an injection of (−)-NPA 0.05 mg kg^{-1} s.c. and demonstrated markedly different levels of activity; 'low activity' responders, i.e. animals giving a count of 10–25 counts 5 min^{-1} were selected for use in the present experiments (for comparison, high activity responders gave counts in the order of 65–80 counts 5 min^{-1}) (see Costall *et al.*, 1983).

Experiments in the marmoset

Stereotaxic techniques Marmosets were anaesthetized with Saffan 1 ml kg^{-1} i.m. (each ml of Saffan contained 9 mg alphaxalone and 3 mg alphadolone acetate) and placed in the stereotaxic frame using squirrel monkey ear bars located in the external auditory meati. Two eye orbit bars were then placed at the base of the orbits to prevent upward movement of the head. Using standard stereotaxic techniques, chronically indwelling guide cannulae (constructed as for the rat) were implanted to allow dopamine infusion into the centre of the nucleus accumbens (Ant. 12.5, Vert. 13.3, Lat. ± 2.0, atlas of Stephan *et al.*, 1980). On recovery from the anaesthetic the discrete size and non-intrusive nature of the implanted guides ensured that animals could be placed together in the holding cages with no interference to the cannulae.

Intracerebral drug infusion Fourteen days after intracerebral cannulation marmosets were re-anaesthetized with Saffan and Alzet osmotic minipumps (previously filled and primed with dopamine as for the rat) were positioned s.c. in the scapula region. The pumps were connected by polythene catheters to injection units which terminated at the centre of the nucleus accumbens as described for the rat. After completion of a 13 day period of infusion the pumps were removed under Saffan-induced anaesthesia.

Behavioural studies Behavioural testing was carried out between 13 h 30 min and 15 h 30 min in a room where temperature and lighting conditions were identical to those of the animal holding rooms. Animals were allowed 1 h to adapt to the new room. The locomotor activity of individual marmosets was assessed using primate cages (76 cm high, 50 cm wide, 60 cm deep) having 4 computer-linked infra-red photocell units placed 7 cm, 23 cm and 53 cm above the cage floor so as to measure activity on or between two perches or on the cage floor. Counts were summated over a 60 min period. Animals were continuously observed via a remote control video camera and videotape recordings taken of all experiments. Analyses of the recordings were undertaken to assess the presence of any behaviour that could interfere with the expression of locomotor hyperactivity, e.g. stereotyped movements, gross excitement, seizures or sedation.

Preselection of marmosets for behavioural experiments Before surgery marmosets were preselected according to their responsiveness to the locomotor stimulant effects of (−)-NPA. Briefly, the marmosets received an injection of (−)-NPA 0.05 mg kg^{-1} s.c. and demonstrated markedly different levels of activity: 'low activity' responders, i.e. animals giving a maximum count of 15–20 counts 10 min^{-1} were selected

for use in the present experiments (for comparison, high activity responders gave counts in the order of 100–130 counts 10 min^{-1}) (see Barnes *et al.*, 1987b).

Histology

On completion of the experiments rats and marmosets were anaesthetized and decapitated, and the brains removed and fixed in formal saline. Brains were frozen and sectioned on a freezing microtome and the sites of drug or vehicle deposition readily identified from the point of termination of the injection cannulae tracks and from the discrete location of the oxidative products for dopamine.

Of the rats prepared for intra-accumbens injection locations were found to be correct in all but three animals where two locations were found to be just anterior to the nucleus accumbens and one posterior in the anterior caudate-putamen complex: these rats had received a dopamine infusion, but failed to respond with hyperactivity and were eliminated from subsequent experiments. Of the rats prepared for infusions/injections into the central area of the amygdala five failed to respond to dopamine infusion into the left subdominant hemisphere. Four of these locations were just lateral to the lateral amygdaloid nucleus, and one was centrally located but just inside the ventral amygdala. Again, these animals failed to respond to dopamine and were excluded from further experimentation. The locations for infusions into the nucleus accumbens of marmoset were all found to be within the defined area. The degree of spread of infusion sites is defined in Figure 9.

Statistical analysis

Results were analysed using one-way or two-way analysis of variance (repeated measure analysis) followed by Dunnett's *t* test.

Drugs

GR38032F (1,2,3,9-tetrahydro-9-methyl-3[(2-methyl-1H - imidazol - 1 - yl) methyl] - 4H - carbazol - 4 - one, HCl.2H_2O) (Chemistry Research Department, Glaxo Group Research Ltd, Ware), 2-methyl-5-hydroxytryptamine (2-Me-5-HT, Chemistry Research Department, Glaxo Group Research Ltd, Ware) (+)-amphetamine sulphate (Sigma) and fluphenazine hydrochloride (Squibb) were prepared in distilled water. Dopamine hydrochloride (Koch light) and (−)-N-n-propylnorapomorphine hydrochloride (Research Biochemicals Inc.) were prepared in a N_2 bubbled solution of 0.1% sodium metabisulphite. (−)-Sulpiride (SESIF) was dissolved in the minimum amount of HCl and neutralized with sodium bicarbonate. Haloperidol (Janssen Pharmaceutica) was prepared in a 1% solution of lactic acid and neutralized with sodium bicarbonate. All doses are expressed as the base and when administered peripherally were given in a volume of 1 ml kg^{-1}.

Results

Abilities of GR38032F, fluphenazine and (−)-sulpiride to inhibit the hyperactivity caused by the injection of amphetamine into the nucleus accumbens of the rat

An increase in locomotor activity followed the bilateral injection of amphetamine (10 μg) into the nucleus accumbens; peak hyperactivity (50 to 60 counts 5 min^{-1}) occurred 20 to 40 min after injection, and declined to control values after 90 to 100 min. Bilateral intra-accumbens injections of GR38032F (0.01–1.0 ng), fluphenazine (0.5–10 ng) or sulpiride (0.1–1.25 ng) given 30 min before the amphetamine caused a dose-related inhibition of the amphetamine effect, GR38032F appearing more potent than fluphenazine or sulpiride (Figure 1). The intra-accumbens injection of the vehicle for GR38032F, fluphenazine or (−)-sulpiride failed to modify the amphetamine effect.

A peripheral pretreatment (30 min i.p.) with GR38032F (0.01–1.0 mg kg^{-1}) or fluphenazine (0.05 and 0.1 mg kg^{-1}) also reduced the hyperactivity caused by the intra-accumbens injection of amphetamine; sulpiride (40 mg kg^{-1}) was also effective but was less potent than either GR38032F or fluphenazine. The inhibitions caused by the central or peripheral administration of the 3 agents were not associated with other overt changes in normal behaviour (Figure 1). The stereotype behaviours and increases in locomotor activity induced by parenteral administration of amphetamine were unaffected by GR38032F, 0.001–1.0 mg kg^{-1} i.p.

Ability of 2-methyl-5-hydroxytryptamine to enhance the hyperactivity caused by the injection of amphetamine into the nucleus accumbens of the rat: inhibition by GR38032F

The hyperactivity caused by amphetamine injected bilaterally into the rat nucleus accumbens was dose-dependently enhanced by 2-Me-5-HT administered into the same nucleus before the amphetamine. The ability of 2-Me-5-HT to enhance the amphetamine response was marked at 1 and 10 ng (Figure 2a). The ability of 2-Me-5-HT to enhance the amphetamine response was dose-dependently inhibited by GR38032F (0.01–1 μg): at the lowest dose, GR38032F effectively returned the hyperactivity response to that exhibited by animals treated with amphetamine alone,

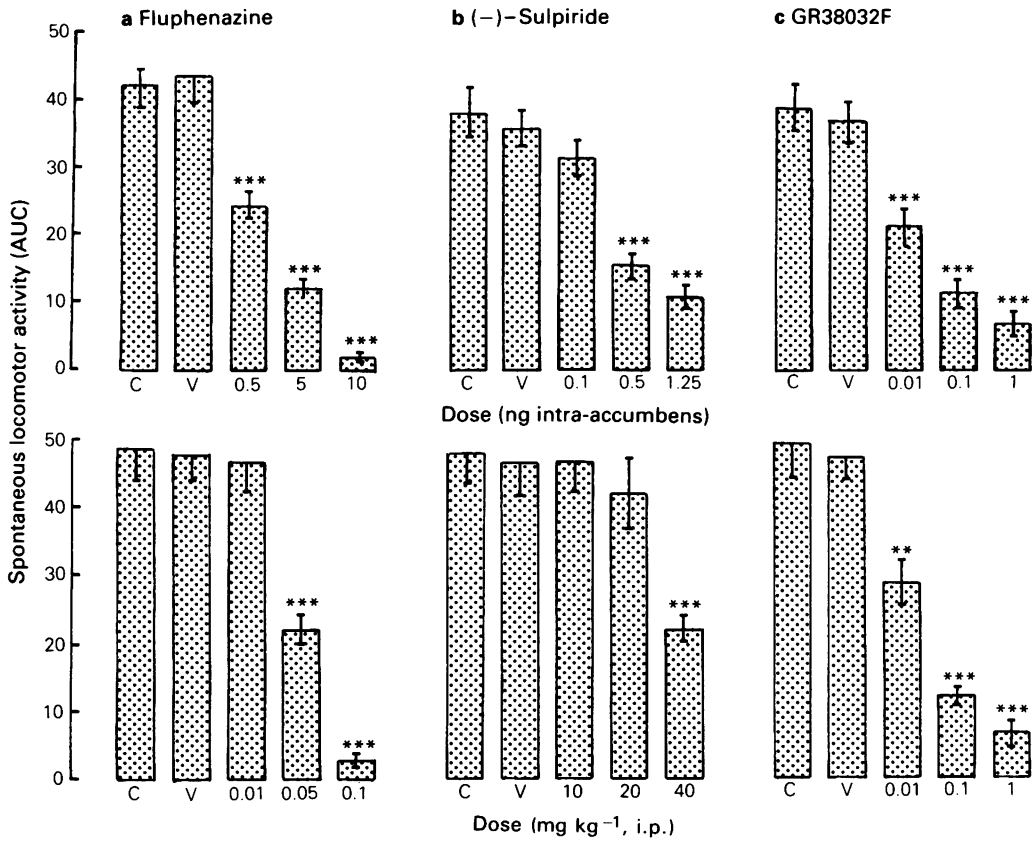

Figure 1 Abilities of (a) fluphenazine, (b) (−)-sulpiride and (c) GR38032F to inhibit the hyperactivity caused by the bilateral injection of amphetamine (10 μg) into the nucleus accumbens of the rat. The control response of animals receiving amphetamine alone is indicated as C, those receiving vehicle before amphetamine as V. Data are given for fluphenazine, (−)-sulpiride or GR38032F administered as a 30 min pretreatment into the nucleus accumbens (ng doses indicated) or peripherally (mg kg^{-1} i.p.). $n = 10$. Vertical lines indicate s.e.means. Area under the curve (AUC) was integrated from the hyperactivity counts for the first 100 min. Significant reductions in the amphetamine response are indicated as $^{**}P < 0.01$, $^{***}P < 0.001$ (one-way analysis of variance followed by Dunnett's t test).

but as the dose of GR38032F was increased not only was the action of 2-Me-5-HT inhibited but also the amphetamine response was diminished (Figure 2b). The intra-accumbens injection of 2-Me-5-HT, 0.01–10 μg, alone had no significant effect on spontaneous locomotor activity.

Abilities of GR38032F and fluphenazine to inhibit the hyperactivity caused by the infusion of dopamine into the nucleus accumbens of the rat

Locomotor hyperactivity in the rat during a 13 day intra-accumbens infusion of dopamine (25 μg 24 h^{-1}) peaked on days 3 and 10. After discontinuation of the dopamine infusion locomotor activity returned to the control values exhibited by animals receiving vehicle infusion (Figure 3).

GR38032F, 0.1 μg kg^{-1} b.d., given throughout the 13 day period of dopamine infusion abolished the first peak of hyperactivity and attenuated the second peak. Higher doses of GR38032F (1.0 and 100 μg kg^{-1} b.d.) abolished both peaks of dopamine-induced hyperactivity but did not depress locomotor activity below the levels of control, vehicle-infused animals. Administration of a lower dose of GR38032F, 0.01 μg kg^{-1} i.p. b.d., failed to modify significantly the dopamine-induced hyperactivity. During treatment with all doses of GR38032F rats remained alert with no evidence of sedation or motor impairment. Furthermore, following the dopamine infusion/GR38032F treatment, the

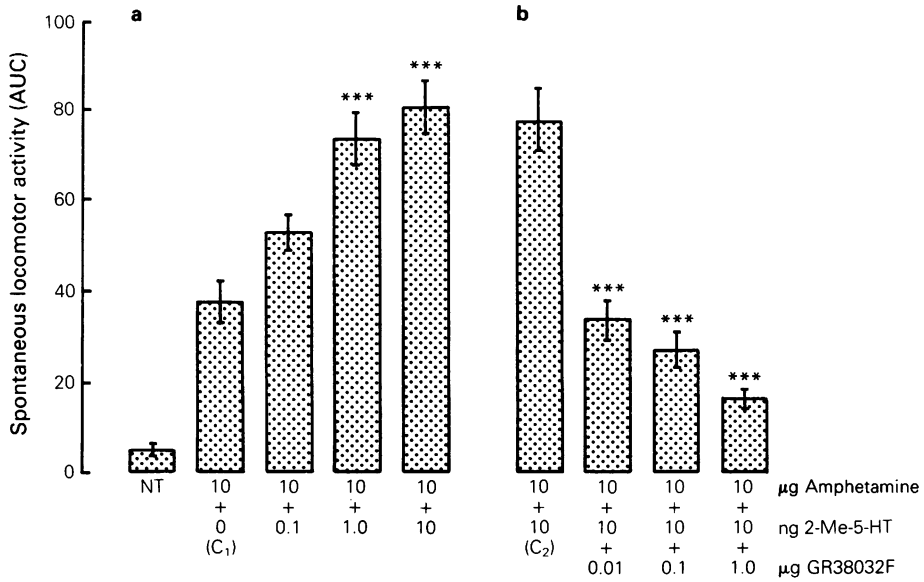

Figure 2 Ability of (a) intra-accumbens 2-methyl-5-hydroxytryptamine (2-Me-5-HT) to enhance the hyperactivity caused by the bilateral infusion of amphetamine (10 µg) into the same nucleus of the rat and (b) the effect of GR38032F to inhibit the effect of 2-Me-5-HT. Animals received no treatment (NT), amphetamine alone (control, C_1), amphetamine plus 2-Me-5-HT or amphetamine plus 2-Me-5-HT combined with GR38032F. 2-Me-5-HT or 2-Me-5-HT plus GR38032F (prepared in the same solution) were injected into the nucleus accumbens 30 min before amphetamine. $n = 6$. Vertical lines indicate s.e.means. Area under the curve (AUC) was integrated from the hyperactivity counts for the first 100 min. A significant enhancement of the amphetamine response (C_1) by 2-Me-5-HT is indicated as $^{***}P < 0.001$ (Dunnett's t test) and (b) significant antagonism of the amphetamine/2-Me-5-HT response (C_2) by GR38032F is indicated as $^{***}P < 0.001$ (Students t test).

level of locomotor activity was not significantly different from control values.

The twice daily administration of fluphenazine (0.01–0.05 mg kg^{-1} i.p.) also reduced the dopamine-induced hyperactivity but the profile of action was not the same as that for GR38032F. Thus a 'high' dose of fluphenazine (0.05 mg kg^{-1} i.p.) not only antagonized the dopamine-induced peaks of hyperactivity but also depressed locomotor activity to levels below control values. Further, following the discontinuation of the dopamine infusion/fluphenazine treatment, animals showed a rebound hyperactivity where the intensity of the response appeared to be related to the dose of fluphenazine employed.

The ability of GR38032F to inhibit the rebound hyperactivity following dopamine infusion into the nucleus accumbens concomitant with haloperidol treatment

Rats receiving an intra-accumbens infusion of dopamine (25 µg 24 h^{-1}) for 13 days and the concomitant administration of haloperidol (0.1 mg kg^{-1} t.d.s.) showed a reduced level of locomotor activity compared to vehicle controls (Figure 4a). A rebound hyperactivity response followed the abrupt discontinuation of the dopamine infusion/haloperidol treatment (Figure 4b) and this was shown to persist for the duration (17 days) of the experiment. Three days after discontinuing the dopamine-haloperidol regime, animals received an acute injection of GR38032F, 0.001, 0.01 or 0.1 mg kg^{-1} i.p., which suppressed the hyperactivity response. The inhibition achieved a maximum effect within 1 or 2 days; thereafter the activity slowly returned to control levels over a period of 3 to 17 days, dependent upon the dose of GR38032F administered.

Effects of GR38032F on the hyperactivity induced by dopamine infused into the left amygdala of rats

In rats with right hemispheric dominance the unilateral infusion of dopamine (25 µg 24 h^{-1}) into the left amygdala for 13 days caused a biphasic increase in locomotor activity with peaks of hyperactivity on days 4 and 9 (Figure 5). The twice daily administration of GR38032F, 100 µg kg^{-1} i.p., inhibited the hyperactivity, reducing the level of response to that exhibited

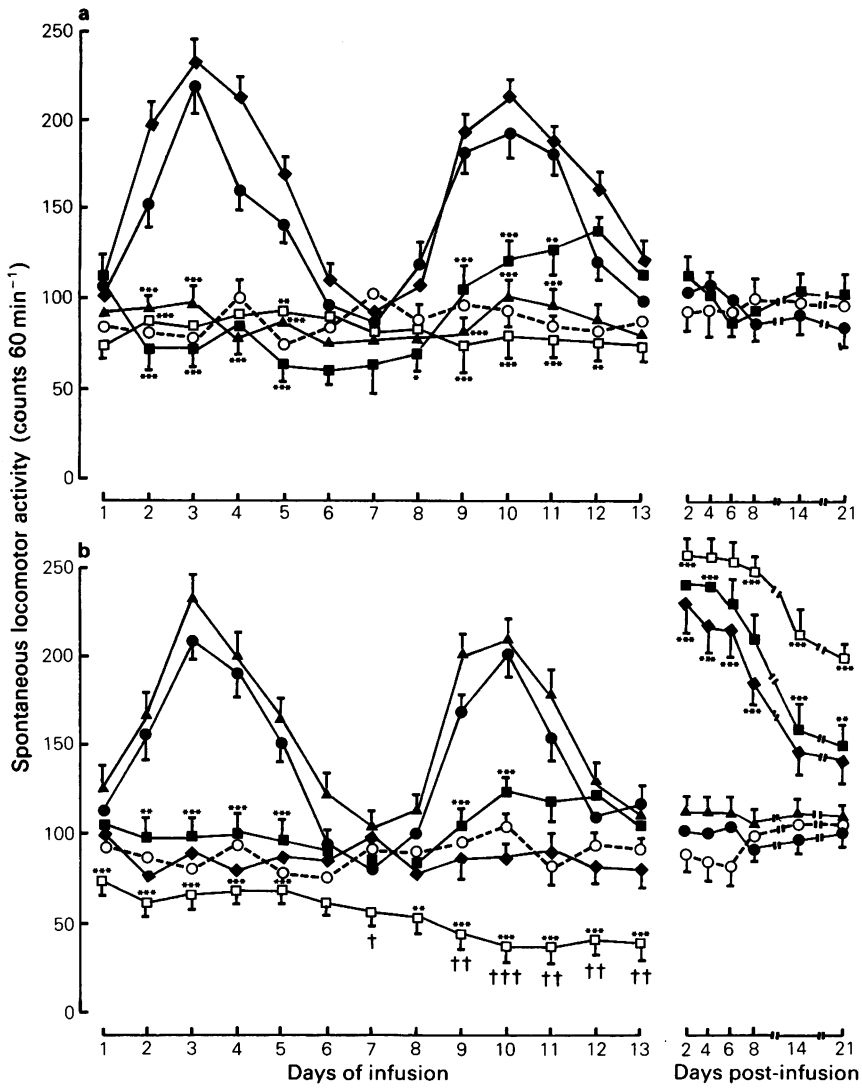

Figure 3 Abilities of (a) GR38032F and (b) fluphenazine to modify the locomotor hyperactivity (measured in photocell cages and expressed as counts 60 min^{-1}) of rats (selected as 'low-activity' responders to challenge with (−)-N-n-propylnorapomorphine) during the 13 days of dopamine infusion, (25 μg 24 h^{-1}, 0.48 μl h^{-1}) into the nucleus accumbens and on days 2–21 after withdrawal of the infusion/drug treatment. (●) Indicates the responses of rats receiving intra-accumbens dopamine (plus vehicle for GR38032F or fluphenazine) and (○) the responses of rats receiving intra-accumbens vehicle for dopamine (plus vehicle for GR38032F or fluphenazine). The responses to dopamine combined with (a) GR38032F (◆) 0.01, (■) 0.1, (▲) 1.0 and (□) 100 μg kg^{-1} i.p. b.d., or combined with (b) fluphenazine (▲) 2.0, (■) 10, (◆) 25 and (□) 50 μg kg^{-1} b.d., are shown $n = 6$. Vertical lines indicate s.e.means. Significant decreases in locomotor activity to below vehicle/dopamine control values are indicated as $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ and significant decreases below vehicle/control values as $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.001$ (for changes during infusion). Significant increases in spontaneous locomotion post-infusion are indicated as $^{**}P < 0.01$, $^{***}P < 0.001$ (two-way analysis of variance followed by Dunnett's t test).

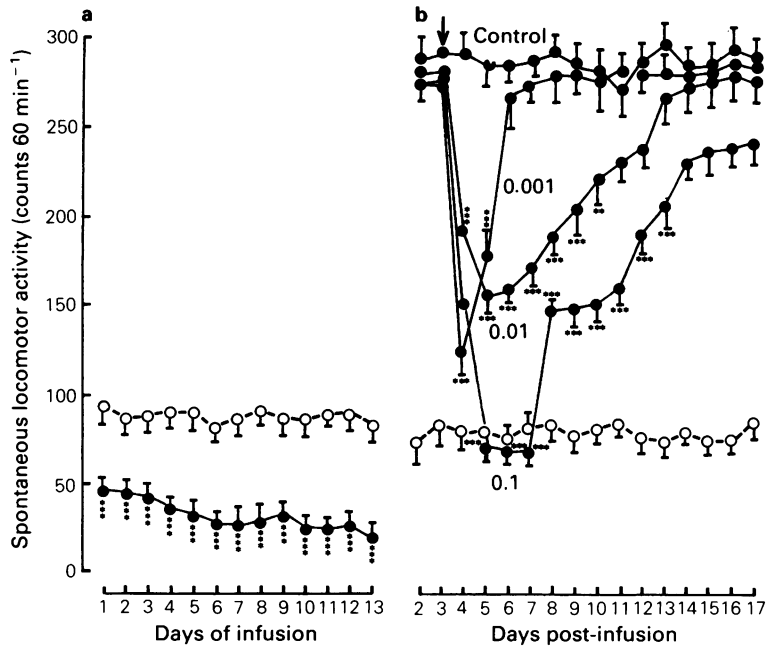

Figure 4 The ability of GR38032F to inhibit the rebound hyperactivity observed following withdrawal of a 13 day period of dopamine infusion into the rat nucleus accumbens combined with haloperidol treatment (0.1 mg kg^{-1} t.d.s., injections at 07 h 00 min, 14 h 00 min, and 23 h 00 min). (a) Shows the spontaneous locomotor activity responses of animals during vehicle infusion into the nucleus accumbens (○), $n = 5$; or dopamine infusion (25 μg 24 h^{-1}) suppressed by haloperidol (●); $n = 20$, divided randomly into 4 groups of 5 for treatments in the post-infusion phase shown in (b). Significant reductions in spontaneous locomotion to below control values are indicated as $^{***}P < 0.001$. In the post-infusion period (b) animals withdrawn from the dopamine/haloperidol treatment were given vehicle for GR38032F (control) or GR38032F, 0.001, 0.01 or 0.1 mg kg^{-1} i.p. (↓ indicates day of injection of GR38032F) given as a single dose. Significant reductions in spontaneous locomotion as compared to control are indicated as $^{**}P < 0.01$, $^{***}P < 0.001$ (two-way analysis of variance followed by Dunnett's t test). The stability of the spontaneous locomotion of animals given vehicle infusion alone into the nucleus accumbens for 13 days is shown for the 17 day post-infusion period (○). Vertical lines indicate s.e.means.

by control vehicle-infused animals. A smaller dose of GR38032F, 1.0 μg kg^{-1} i.p. b.d., had a similar effect. The lower dose of GR38032F (0.1 μg kg^{-1} i.p.) given twice daily reduced the first hyperactivity peak but failed to reduce consistently the second peak (Figure 5a).

In a second experiment, the 13 day infusion of dopamine into the left amygdala was again shown to enhance locomotor activity although there was no clear biphasic pattern of responding (Figure 5b). The concomitant administration of fluphenazine, 50 μg kg^{-1} b.d., abolished the hyperactivity response; a slightly higher dose of 100 μg kg^{-1} given twice daily not only inhibited the dopamine-induced hyperactivity but also reduced the level of activity to values below those of the vehicle-infused control animals. Fluphenazine (25 μg kg^{-1} b.d) antagonized the dopamine-induced hyperactivity during the first 6 days, but subsequently hyperactivity developed and persisted for the remainder of the infusion period.

Ability of GR38032F given unilaterally into the central amygdala to modify the hyperactivity induced by dopamine infused into the left amygdala of rats

Rats with right hemispheric dominance and given an infusion of dopamine (25 μg 24 h^{-1}) into the left amygdala exhibited peaks of hyperactivity with the most intense responses occurring on days 3 and 9. On these days animals were given a single injection of GR38032F (0.1–100 ng) or fluphenazine (0.05–0.5 ng) into the amygdala either on the same side (and therefore at the same site) as the infusion of dopamine or on the non-infused side.

The injection of GR38032F in doses as low as 0.1 ng into the non-infused side caused a significant reduc-

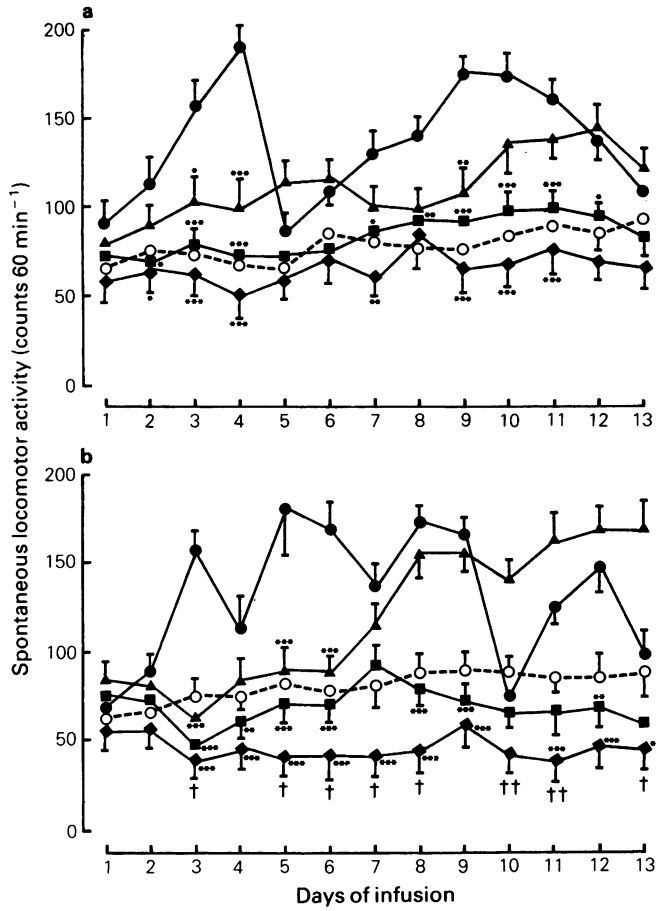

Figure 5 Abilities of (a) GR38032F and (b) fluphenazine to inhibit the locomotor hyperactivity caused by an infusion of dopamine (25 μg 24 h^{-1}) for 13 days into the left amygdala of rats having right hemispheric dominance (assessed in a turn preference test). Animals received the infusion of dopamine (plus vehicle for GR38032F or fluphenazine) (●), vehicle for dopamine (plus vehicle for (a) GR38032F or (b) fluphenazine) (○) or (a) dopamine plus GR38032F 0.1 (▲) 1.0 (■) 25 and 10 (◆) μg kg^{-1} i.p. b.d. (for 13 days) or (b) dopamine plus fluphenazine 25 (▲), 50 (■) or 100 (◆) μg kg^{-1} b.d. (for 13 days). $n = 6$. Vertical lines indicate s.e.mean. Significant decreases in locomotor activity to below vehicle/dopamine control values are indicated as $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ and significant decreases below vehicle/control values as $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ (two-way analysis of variance followed by Dunnett't t test).

tion in the dopamine response, while higher doses of at least 10 ng were required to be given into the infused side to reduce significantly dopamine-induced hyperactivity (Figure 6). Fluphenazine (0.025–0.5 ng) was more potent than GR38032F in reducing the hyperactivity caused by dopamine infusion, and had a similar potency when injected into either the dopamine-infused or non-infused side (Figure 6). The injection of GR38032F or fluphenazine into the amygdala was not associated with the development of effects that could non-specifically interfere with locomotor activity.

Ability of GR38032F to modify the hyperactivity caused by the infusion of dopamine into the nucleus accumbens of the marmoset

The bilaterial infusion of dopamine (25 μg 24 h^{-1}) into the nucleus accumbens of the marmoset for 13 days caused a 4 fold increase in activity which peaked on the 5th to 7th days of infusion, the response being maintained for a further 4 or 5 days before declining to control (vehicle infused) levels (Figure 7).

The peripheral administration of GR38032F (1–10 μg kg^{-1} i.p. b.d.) during the period of dopamine

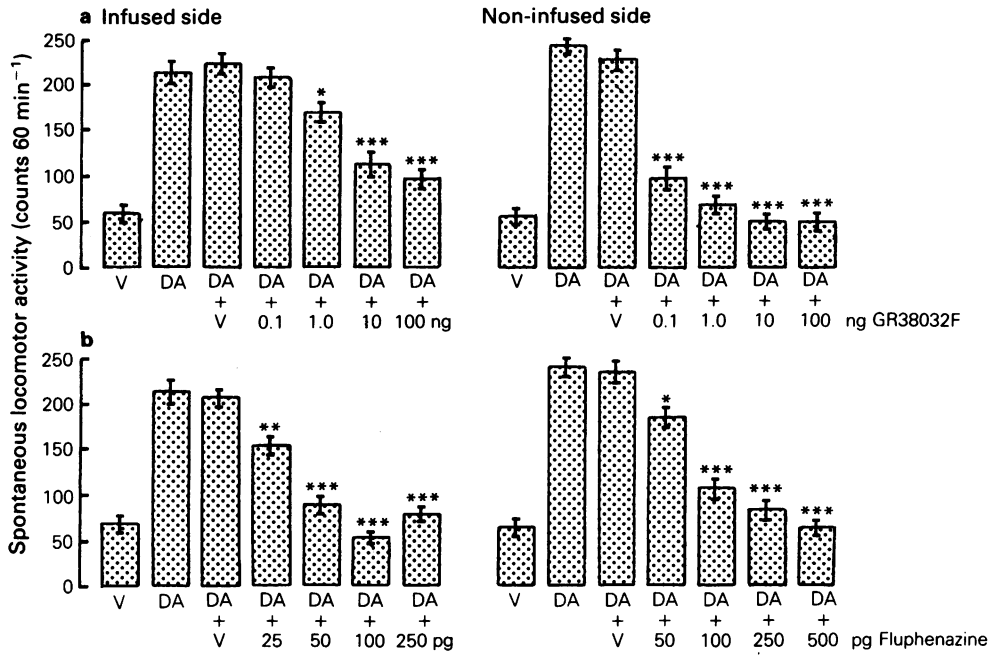

Figure 6 Ability of acute injections of (a) GR38032F and (b) fluphenazine into the amygdala, right or left side, to inhibit the hyperactivity caused by 25 μg 24 h^{-1} of dopamine infused unilaterally into the left amygdala when the right hemisphere was dominant (assessed in a turn preference test). The left amygdala was designated 'infused side', the right amygdala the 'non-infused side'. Testing was carried out on days 3 and 9 of the infusion of dopamine when the peak hyperactivity, in the order of 250 counts 60 min^{-1}, was recorded (indicated as DA or DA + V). Vehicle for GR38032F or fluphenazine, injected into the infused or non-infused side, failed to modify the hyperactivity induced by dopamine (DA + V). Significant reductions in the responses to dopamine by GR38032F or fluphenazine are indicated as $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ (one-way analysis of variance followed by Dunnett's t test); control responses of animals receiving the infusion of vehicle for dopamine, indicated as V, were indistinguishable from those of non-infused rats. Values are the mean of 6 determinations where the vertical lines represent the s.e.means.

infusion reduced the dopamine-induced hyperactivity, but locomotor activity levels were never reduced to below control levels. After discontinuation of the dopamine infusion/GR38032F treatment regimen, the levels of locomotor activity remained indistinguishable from those of vehicle-infused animals (Figure 7). Fluphenazine (0.01–2.5 mg kg^{-1} i.p. b.d.) also inhibited the dopamine-induced hyperactivity, but the levels of activity were markedly reduced to values below control. Further, after discontinuation of the dopamine infusion/fluphenazine treatment regimen, animals that had received the highest dose of fluphenazine (2.5 mg kg^{-1}) showed a significantly enhanced level of locomotor activity during the first 4 post-infusion days and during the 2nd week (Figure 7).

Histology

The infusion sites for dopamine within the amygdaloid complex of the rat were found in the area of the central or lateral amygdaloid nuclei; representative data from 30 animals are shown in Figure 8a. The spread of the locations for vehicle infusions or drug and vehicle injections into the amygdala were indistinguishable from those found for dopamine. The injection or infusion sites for dopamine or drugs directed to the nucleus accumbens of the rat were found to be located within this nucleus; representative data from 15 animals are shown in Figure 8b. The sites for the infusion of dopamine or vehicle into the nucleus accumbens of the marmoset were found to be located within the nucleus accumbens; representative data obtained from 15 animals are shown in Figure 9.

Discussion

The injection of dopamine agonists or the infusion of dopamine into the nucleus accumbens of the rat brain will enhance locomotor activity (Pijnenburg & Van

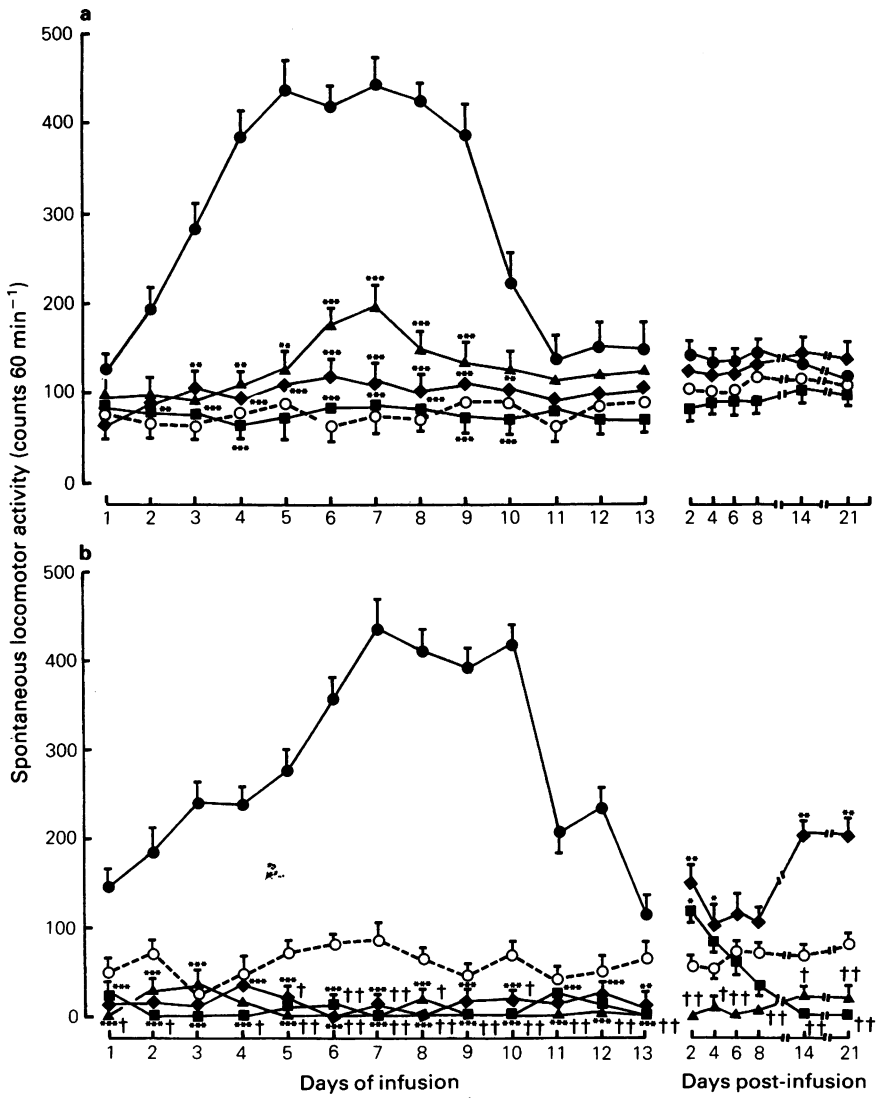

Figure 7 Abilities of (a) GR38032F and (b) fluphenazine to modify the locomotor hyperactivity of marmosets (selected as 'low activity' responders to challenge with (−)-N-n-propylnorapomorphine) during 13 days of infusion with dopamine (25 μg 24 h^{-1}, 0.48 μl h^{-1}) into the nucleus accumbens and on days 2 to 21 after withdrawal of dopamine infusion/drug treatment. (●) Indicates the responses of marmosets receiving intra-accumbens dopamine (plus vehicle for (a) GR38032F or (b) fluphenazine) and (○) the responses of marmosets receiving intra-accumbens vehicle for dopamine (plus vehicle for (a) GR38032F or (b) fluphenazine). The responses to dopamine combined with (a) GR38032F (▲) 0.1, (■) 1.0 and (◆) 10 μg kg^{-1} i.p. b.d., or (b) fluphenazine (▲) 0.01, (■) 0.1 and (◆) 2.5 mg kg^{-1} b.d. are shown. $n = 6$. Vertical lines indicate s.e.mean. Significant decreases in locomotor activity to below vehicle/dopamine control values are indicated as $^{**}P < 0.01$, $^{***}P < 0.001$ and significant decreases below vehicle/control values as $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ (for changes during infusion). Significant increases in spontaneous locomotion post-infusion are indicated as $^{**}P < 0.05$, $^{***}P < 0.01$, and decreases as $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ (two-way analysis of variance followed by Dunett's t test).

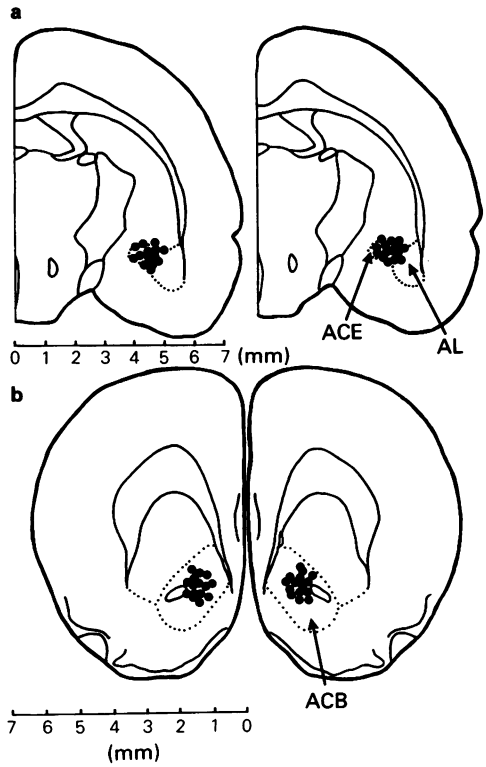

Figure 8 Diagrammatic representation of the sites of dopamine infusion (●) into (a) the amygdala or (b) the nucleus accumbens of the rat. Representative data from 30 brains are given for the unilateral infusions into the amygdala and from 15 brains for the bilateral infusions into the nucleus accumbens. Co-ordinates are according to De Groot (1959). ACE-nucleus amygdaloideus centralis, AL-nucleus amygdaloideus lateralis and ACB-nucleus accumbens septi.

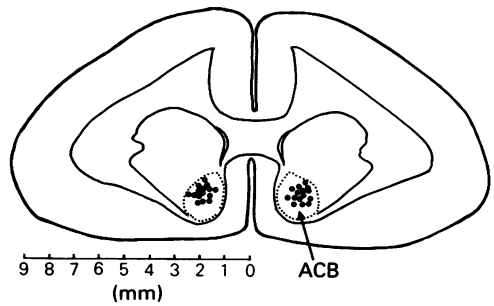

Figure 9 Diagrammatic representation of the sites of bilateral infusion of dopamine (●) into the nucleus accumbens (ACB) of the marmoset. For clarity of presentation representative data from 15 brains only are shown. The co-ordinate is according to Stephan *et al.* (1980).

Rossum, 1973; Jackson *et al.*, 1975; Costall *et al.*, 1976b; 1983). In the present study it was found that GR38032F, a potent and highly selective antagonist at the 5-HT_3 receptor (Brittain *et al.*, 1987), inhibited the hyperactivity induced by an acute intra-accumbens injection of amphetamine in the rat or by persistent intra-accumbens infusion of dopamine in the rat or marmoset. Since GR38032F has no known affinity for other neurotransmitter receptor sites and, in particular, does not interact with dopamine receptors (Brittain *et al.*, 1987), the inhibitory effect of GR38032F probably reflects 5-HT_3 receptor blockade. This is likely to occur within the nucleus accumbens since the injection of GR38032F into this area reduced the response to the intra-accumbens injection of amphetamine. The inhibition by peripherally administered GR38032F of the hyperactivity caused by the intra-accumbens injection of amphetamine or infusion of dopamine may also reflect an action within the nucleus accumbens, although additional effects in other brain regions cannot be ruled out (see below). The failure of GR38032F to inhibit the responses to parenteral amphetamine was not unexpected. Amphetamine given parenterally induces stereotyped behaviour and increases locomotor activity by acting on catecholamine systems in both limbic and striatal areas. Since GR38032F has no effect on striatal dopamine related behaviours (Costall *et al.*, 1987), it would not be expected to reduce these effects.

The ability of GR38032F to reduce locomotor hyperactivity, while having no overt effect on normal behaviour, indicates that 5-HT may exert a facilitatory role on dopaminergic transmission. This has further support from the findings that injections of 5-HT into the nucleus accumbens increases locomotor activity (Pijnenburg *et al.*, 1975; Costall *et al.*, 1979; Makanjuola *et al.*, 1980). It could be envisaged that the antagonism of a facilitatory effect is sufficient in its own right to reduce dopamine-mediated hyperactivity. Further evidence that facilitation of the dopamine hyperactivity response by 5-HT is mediated via the 5-HT_3 receptor is obtained from the use of the selective 5-HT_3 receptor agonist 2-methyl-5-HT. Intra-accumbens injections of 2-methyl-5-HT increased the hyperactivity response induced by amphetamine and this effect was antagonized by GR38032F. The effect of intra-accumbens 2-methyl-5-HT was always to enhance a developed hyperactivity while having no direct effect on spontaneous locomotor activity when given alone. These data are consistent with the view that 5-HT, via the 5-HT_3 receptor, has a modulatory function which is only apparent when the mesolimbic dopamine system is disturbed. However, injections of 5-HT into the nucleus accumbens can also reduce locomotor activity

(Costall *et al.*, 1976a; Carter & Pycock, 1978; Jones *et al.*, 1981) and blockade of a 5-HT facilitatory effect with 5-HT antagonists may allow a dominance of a 5-HT inhibitory system. An alternative explanation for the observed inhibitory effect of injected 5-HT may be that 5-HT can exert a preferential action on autoreceptors situated on 5-HT nerve terminals in the nucleus accumbens.

The observation that GR38032F can reduce a raised mesolimbic dopaminergic activity is not restricted to the nucleus accumbens, but can also be demonstrated in the amygdala. The unilateral infusion of dopamine into the central nucleus of the amygdala leads to increased locomotor activity but this response is restricted to the left amygdala when the right hemisphere is dominant, as assessed in a turn preference test (Bradbury *et al.*, 1985). Neuroleptic agents given parenterally are known to antagonize the hyperactivity caused by dopamine infusion into this site (Bradbury *et al.*, 1985). In the present study, fluphenazine antagonized the hyperactivity when injected into either the left or the right amygdala. This result indicates the importance of interhemispheric communication in the regulation of locomotor activity in the limbic system and the present studies provide important evidence of a 5-HT involvement with this response. Thus, the injection of GR38032F into either the right or left amygdala of rats (receiving dopamine infusion into the left amygdala) inhibited the dopamine-induced hyperactivity response. An inhibition was also recorded following the peripheral injection of GR38032F (and fluphenazine) which may involve an action in the amygdala of either hemisphere, and in other limbic areas. These observations of drug effects on the consequences of dopamine excess in the amygdala provide a second body of evidence that, at a behavioural level, GR38032F can effect a response similar to that of a dopamine antagonist.

However, whilst the present results indicate that neuroleptic agents such as fluphenazine, haloperidol and sulpiride have a similar spectrum of inhibitory action to GR38032F on raised mesolimbic dopaminergic activity, there were also important differences. The first distinction was the failure of GR38032F either to reduce locomotor activity in normal animals or to inhibit raised limbic dopaminergic activity to levels below control values. This contrasts markedly with the depressant effects of neuroleptic agents which indiscriminately depress all forms of motor responses. The absence of indiscriminate depressant activity is an important property of GR38032F and indicates that this agent may have particular value in restoring disturbed dopaminergic activity to normal. The second distinction relates to the long-term consequences of a sub-chronic administration of neuroleptic agents in the nucleus accumbens-dopamine infusion model. Discontinuation of the neuroleptic/dopamine treatments resulted consistently in marked and prolonged increases in locomotor activity, which may reflect changes in dopamine receptor sensitivity (see Rupniak *et al.*, 1983). In contrast, discontinuation of the GR38032F/dopamine treatments did not cause increased locomotor activity and values remained at control levels.

The failure of GR38032F to cause a 'rebound' response, and the concept that such a response to classical neuroleptics may compromise the therapeutic potential of GR38032F if administered after chronic neuroleptic treatment, prompted subsequent experiments to determine the effect of GR38032F on the hyperactivity following the discontinuation of an intra-accumbens dopamine/haloperidol treatment. In these experiments, GR38032F markedly and dose-dependently reduced the 'rebound' hyperactivity response. Surprisingly, single low doses of GR38032F not only reduced the response but the effect persisted for several days before locomotor activity returned to the 'rebound' high level. These data are indicative of a 5-HT$_3$ involvement in the modulation of enhanced locomotor responding.

The persistent effect of GR38032F is more difficult to explain. The half-life of GR38032F in plasma in the rat is less than 4 h (Bell, personal communication) and so it is unlikely that an effect which lasts for several days can be attributed to a continuing drug effect. It is possible that 5-HT perpetuates a facilitatory effect on dopaminergic transmission via a 5-HT-dopamine-5-HT loop. A break in the loop, by antagonism of 5-HT$_3$ or dopamine reeptors, leads to reduced hyperactivity. But the initiating factor for hyperactivity (presumably dopamine receptor supersensitivity) is unaffected and there is a gradual return to the 'rebound' state.

Further direct evidence to support the proposed modulatory role of 5-HT$_3$ receptors on mesolimbic dopaminergic transmission comes from the work of Hagan *et al.* (1987). In these experiments the hyperactivity response and increased dopamine turnover in the nucleus accumbens caused by injections of the stable neurokinin analogue, di-Me-C7, into the ventral tegmental area of rats were both inhibited by GR38032F. In addition, we have shown that other selective 5-HT$_3$ antagonists, including ICS 205-930, are effective in these models of mesolimbic overactivity (unpublished observations).

The above findings may have clinical implications. If it is accepted that raised dopaminergic activity within the limbic brain areas contributes to the development of schizophrenia, and there is evidence that this may occur in the amygdala (Reynolds, 1983), then the reduction of limbic dopaminergic 'overactivity' by GR38032F in the animal models may indicate a potential antipsychotic action. In turn, this would indicate that 5-HT may be involved in the aetiology of schizophrenia.

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