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Actions of ORG 5222 as a Novel Psychotropic Agent

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COSTALL, B., A. M. DOMENEY, M. E. KELLY, R. J. NAYLOR AND D. M. TOMKINS. *Actions of ORG 5222 as a novel psychotropic agent.* PHARMACOL BIOCHEM BEHAV **35**(3) 607–615, 1990.—ORG 5222 is a tetracyclic compound with high affinity for dopamine and 5-HT_2 receptors. ORG 5222 was compared to fluphenazine in behavioural tests and was shown to be less potent to cause catalepsy on peripheral administration or to induce asymmetric body posturing following intrastriatal injection. On injection into the nucleus accumbens, ORG 5222 antagonised spontaneous and amphetamine-induced hyperactivity. The peripheral administration of ORG 5222 antagonised the hyperactivity induced by infusion of dopamine into the nucleus accumbens of rat or ventral striatum of the marmoset and, unlike the use of fluphenazine, there was no evidence of a 'rebound' hyperactivity after discontinuation of treatment. Furthermore, ORG 5222 prevented changes in responsiveness to dopamine agonist challenge following dopamine infusion. In a mouse black and white test box and the rat elevated plus maze ORG 5222 released exploratory behaviour suppressed by the aversive white or elevated environments. It is concluded that ORG 5222 is effective to antagonise mesolimbic dopamine function in the rodent and primate and an aversive behaviour in rodent tests. Such effects reveal a novel profile of action of ORG 5222 in behavioural paradigms predictive of antipsychotic and anxiolytic potential and may relate to a dopamine and 5-HT receptor antagonism.

ORG 5222 Dopamine and 5-HT receptor antagonist Neuroleptic agent Anxiolytic profile

MIANSERIN is a tetracyclic compound used in the treatment of depression. It has high affinity for alpha-adrenoceptors, histamine and 5-hydroxytryptamine receptors of the 5-HT_2 subtype, with little affinity for dopamine receptors (21, 26, 29). However, modification of the tetracyclic skeleton has been shown to confer additional affinity for dopamine and other receptors (24) and a combination of such properties may account for the varying abilities of tetracyclic pyrrolidine and piperidino compounds to induce changes in behaviour characteristic of a neuroleptic agent. Thus, a reduced ability to induce catalepsy in animals may reflect a reduction in serotonergic function (2, 9, 24), yet such actions may also contribute to an antipsychotic potential and broaden the therapeutic usefulness of such agents to the treatment of anxiety (3, 4, 18).

There is a clear need to develop antipsychotic agents which lack the extrapyramidal and other side effects of neuroleptic agents, and the tetracyclic neuroleptic agents which are structurally related to mianserin may present as one such group. In this study we investigate in a series of behavioural experiments the profile of action of ORG 5222 (trans-5-chloro-2,3,3a,12b-tetrahydro-2-methyl-1H-dibenzo[2,2:6,7]oxepino[4,5-C]pyrrole) (Fig. 1), which preliminary studies have shown to be a potent dopamine and 5-hydroxytryptamine receptor antagonist with a potential antipsychotic action (24).

METHOD

Animals and Housing

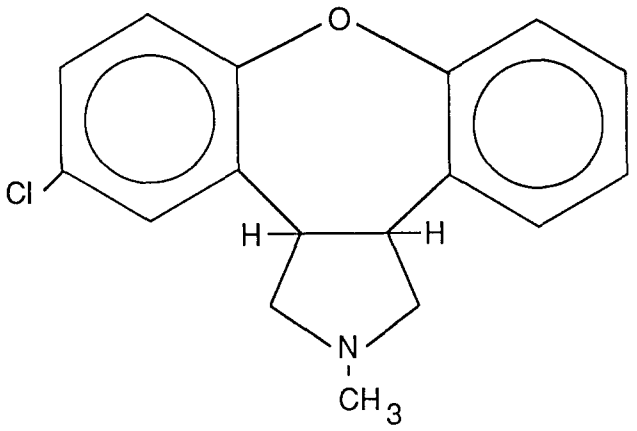
Male albino B.K.W. mice (Bradford strain) weighing 25–30 g were used throughout the studies. Mice were housed in groups of 10 in conditions of constant temperature (21°C) and controlled lighting (dark period 07.00–19.00 hr) and fed ad lib on a standard laboratory chow.

Female Sprague-Dawley (CD) Bradford strain rats weighing 300 ± 25 g were used throughout the experiments excepting in the Elevated Plus Maze test where male Hooded Lister rats (250–300 g) obtained from OLAC were used. Animals were housed in groups of 5 at a temperature of 21 ± 2°C on a 12-hr light-dark cycle (dark period 20.00–08.00 hr) and fed CRM diet (Labsure) and allowed water ad lib.

Marmosets were housed two per cage and allowed food (Mazuri primate diet, S.D.S. Ltd. Essex) and water ad lib. 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 hr with a 12 hr dark cycle, with lights on between 07.00–19.00 hr. Simulated dawn and twilight periods were achieved using a single 60-W bulb illuminated 0.5 hr before and after the main lights came on and went off respectively. During the 12-hr dark period a single 60-W red bulb was illuminated to avoid complete darkness.

Stereotaxic Surgery, Cannula Implantation and Intracerebral Drug Injection or Infusion

Studies using rats. Animals were subject to standard stereotaxic techniques for the implantation of chronically indwelling



ORG 5222

FIG. 1. The structure ORG 5222.

guide cannulae for subsequent bilateral intracerebral infusion of dopamine or injection of drug at the centre of the nucleus accumbens (ant. 9.4, vert. 0.0, lat. ±1.6) according to the atlas of de Groot (17). Implanted guides were kept patent for a 14-day recovery period using stainless steel stylets. After the recovery period rats received an intracerebral injection of ORG 5222 or fluphenazine and amphetamine, or vehicle 1 μl administered over 5 sec, the injection unit remaining in position for a further 55 sec before being withdrawn and the stylets replaced. Other animals were anaesthetised with halothane (using an N_2O/O_2 carrier) for subsequent implantation of two Alzet osmotic minipumps into the scapula region. The pumps were each attached via polythene tubing catheters to stainless steel injection units which were made to fit permanently into the previously implanted guides in place of stylets, but terminating in the centre of the nucleus accumbens. The pumps had been previously filled with a solution of dopamine (2.17 μg/μl) or its solvent and delivered the solution at a rate of 0.48 μl/hr for 13 days. Following behavioural assessments on day 13 the pumps were removed under halothane anaesthesia. For fuller details of stereotaxic surgery and pump implantation see (5).

Studies using marmosets. Marmosets were subject to standard stereotaxic techniques for the implantation of chronically indwelling guide cannulae for infusion of dopamine in the ventral striatum (ant. 12.5, vert. 13.3, lat. ±2.0) according to the atlas of Stephan and colleagues (28). Implanted guides, constructed of stainless steel and held in perspex blocks, were located 2 mm below the dura; stylets constructed of stainless steel were placed into the guide cannulae and the animals allowed a minimum of 14 days recovery. After the recovery period, marmosets were anaesthetised with Saffan (Glaxovet Ltd.) for the subcutaneous implantation in the scapula region of two Alzet osmotic minipumps for the infusion of dopamine or vehicle into the ventral striatum. The pumps were attached to indwelling injection units via polythene catheter tubing. After completion of the 13-day infusion, marmosets were reanaesthetized with Saffan for the removal of pumps. For full details of stereotaxic surgery and pump implantation see (1).

Behavioural Tests

Catalepsy induction. Catalepsy was assessed according to the method of Costall and Naylor (14). Briefly, rats were placed in the observation/testing cages 30 min before treatment with ORG 5222 or fluphenazine to allow adaptation to the new environment. Animals were tested by placing both front limbs over a 10 cm high horizontal bar, a cataleptic animal maintaining this position for a period of time dependent upon the degree of catalepsy. Animals were tested frequently after drug administration to determine the onset of catalepsy and the intensity of catalepsy was then measured at 30-min intervals throughout the duration of the drug effect. In order to account for animals maintaining the imposed position for an infinite period of time the following scoring system was adopted for estimation of the intensity of catalepsy. Intensity (min) of 0, 0.1–2.5, 2.6–5.0, 5.1–10.0, 10.1–20 and 20.1–∞ was scored 0, 1, 2, 3, 4 and 5 respectively.

Asymmetric body posturing following unilateral intrastriatal drug injection. Asymmetric body posturing was measured according to the method of Costall and colleagues (11). Briefly, rats received an injection of ORG 5222 or fluphenazine into one striatum and vehicle injection into the other striatum. After 30 min animals were challenged with apomorphine (0.25 mg/kg SC) and the development of an asymmetric body posturing recorded over the duration of apomorphine effect (45 min). The maximum response to apomorphine occurred after 15 min and the asymmetric body posture was scored according to observations of responding in the open field and to handling on a 0–3 scoring system where 0 = no asymmetry, response of animals the same as untreated rats, 1 = a distinct tendency for animals to move in one direction when handled, but still capable of movement in either direction, 2 = spontaneous movements in one direction, a twisting of the body in this direction, exaggerated when handled, with inability to move in opposite direction, 3 = a marked and intense twisting of the body, active circling movements when disturbed, the animal being unable to move in the opposite direction.

Drug action to modify spontaneous or amphetamine-induced hyperactivity. Locomotor activity was measured in cages equipped with a photocell unit [see (5)]. Rats received a bilateral injection of ORG 5222, fluphenazine or vehicle into the nucleus accumbens and were placed immediately into the activity cage for measurement of changes in spontaneous locomotor activity over a 30-min period. In other experiments rats received ORG 5222, fluphenazine or vehicle followed after 30 min by amphetamine (10 μg). Animals were placed immediately into the activity cage and changes in locomotor activity were recorded for the duration of the amphetamine-induced effect.

Drug action to modify hyperactivity induced by dopamine infusion into the rat nucleus accumbens. Animals were preselected according to their responsiveness to the locomotor stimulant effects of the dopamine agonist (−)N-n-propyl norapomorphine [(−)-NPA]. Briefly, rats received an injection of (−)-NPA 0.05 mg/kg SC and demonstrated markedly different levels of activity; 'low activity' responders, i.e., animals giving a count of 10–25 counts 5 min were selected for use in the present experiments (for comparison, high activity responders gave counts in the order of 65–80 counts 5 min) [see (5)].

Selected animals received a 13-day bilateral infusion of dopamine (25 μg/24 hr, 0.48 μl/hr) or vehicle into the nucleus accumbens, and concomitant IP b.i.d. administrations of ORG 5222, fluphenazine or vehicle. Changes in locomotor activity were recorded during the 13-day period and for 7 weeks postinfusion. In some experiments the ability of such treatments to modify the responsiveness to (−)NPA challenge was assessed during the postinfusion period.

Drug action to modify hyperactivity induced by dopamine infusion into the ventral striatum of the marmoset. Behavioural testing was carried out between 08.30 and 11.30 a.m. The locomotor activity of marmosets was assessed using individual primate cages (76 cm high, 50 cm wide, 60 cm deep) having 4 computer-linked infra-red photocell units placed 7, 23 and 53 cm above the floor of the cage in such a manner as to measure movement on or between two perches or on the cage floor. Counts

were summated over a 60-min period. At all times the animals were observed through a remote control video camera and video recordings were 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, sedation. At the completion of the experiment the marmoset were returned to the holding rooms.

Marmosets received a 13-day bilateral infusion of dopamine (25 μg/24 hr, 0.48 μl/hr) or vehicle into the ventral striatum and concomitant IP twice daily administrations of ORG 5222 or vehicle. Changes in locomotor activity were recorded during the 13-day infusion period.

In other experiments marmosets were initially selected on the basis of their response to the locomotor stimulant effects of (−)NPA (0.05 mg/kg SC) into 'low,' 'moderate' and 'high' activity responders. 'High' activity responders, i.e., animals recording 350–400 counts/10-min period were selected for further use and received a 13-day dopamine or vehicle infusion into the ventral striatum and concomitant IP b.i.d. administrations of ORG 5222, fluphenazine or vehicle. The responsiveness of the treatment groups to the locomotor stimulant effects of (−)NPA was assessed in the post infusion period.

Modification of mouse behaviour in the black:white test box. Tests for changes in behaviour which are known to be influenced by anxiolytic agents were conducted between 13.00 and 18.00 hr in a quiet darkened room illuminated with a red light. Mice were taken from a dark holding room in a dark container to the dark testing room where, after a 1-hr period of adaptation to the new environment and 30 min after pretreatment with ORG 5222 or vehicle, mice were placed individually into the centre of the white brightly lit area of the test box. The apparatus used for the detection of changes in exploratory behaviour consisted of an open-topped box (45 × 27 × 27 cm high) lined into 9 cm squares, two-fifths painted black and illuminated under a dim red light (1 × 60 W) and partitioned from the remainder of the box which was painted white and brightly illuminated with a 60-W light source located 17 cm above the box. An opening 7.5 × 7.5 cm located at a floor level in the centre of the partition allowed access between the two compartments. The mice were observed over a 5-min period by remote video-recording and four behaviours noted: 1) the number of exploratory rearings in the white and black sections; 2) the number of line crossings in the white and black areas; 3) the time spent in the white and black areas; and 4) the latency of the initial movement from the white to black area. Mice were used once only in treatment groups of five.

Elevated plus maze test in rats. Tests were conducted between 10.00 and 14.00 hr in an illuminated room using a methodology based on the model of Handley and Mithani (20) and modified by Costall and colleagues (13). Rats were transferred to the experimental room at least 1 hr before testing. The apparatus consisted of an X-shaped maze constructed of perspex, elevated 70 cm from the floor and comprised of two (opposite) closed arms and two open arms. The arms were 45 cm long and 10 cm wide. The closed arms had sides 10 cm high while the open arms had no sides. The floor was covered with rubber matting and lined so that each arm was divided into two equal sections. The 10-min test period commenced by placing a rat on the centre square (all facing the same open arm) and the number of entries into and time spent in the furthermost sections of both the open and closed arms was recorded.

Histological Analysis of Rat and Marmoset Brains

On completion of experiments cannulated animals were killed and the brains removed and fixed in formal saline. Brains were frozen and sectioned on a freezing microtome and the sites of deposition of dopamine or vehicle identified from the termination of the guide cannulae tracks. The location of the sites of drug or vehicle deposition was found to be within the area of the nucleus accumbens and ventral striatum, and indistinguishable from those reported in our previous studies (7).

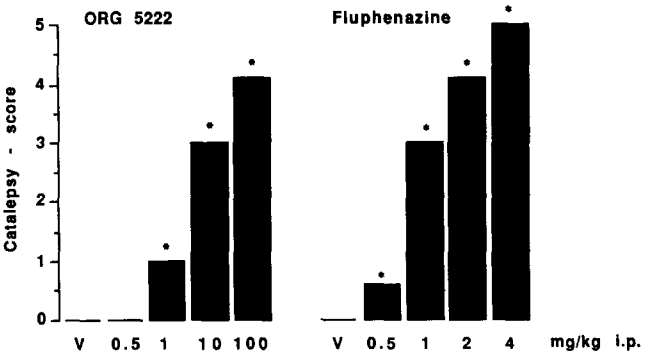


FIG. 2. Cataleptic responses to ORG 5222 and fluphenazine in the rat. The intensity of catalepsy was assessed on a 0 to 5 scoring system as detailed in the text. Each value is the mean of 6 determinations. Standard errors on all values are less than 13.2% of the means. A significant difference compared to vehicle-treated controls is indicated *$p<0.05$ (one-way ANOVA followed by Dunnett's t-test).

Drugs

Apomorphine·HCl (Macfarlan Smith) and dopamine·HCl (Koch Light) were prepared in nitrogen bubbled distilled water containing 0.1% sodium metabisulphite. ORG 5222 (trans-5-chloro-2,3,3a,12b-tetrahydro-2-methyl-1H-dibenzo[2,3:6,7]oxepino[4,5-c]pyrole) (Organon), fluphenazine·HCl (Squibb) and d-amphetamine SO_4 (Sigma) were dissolved in distilled water. Doses of drugs are expressed as the base and administered peripherally in a

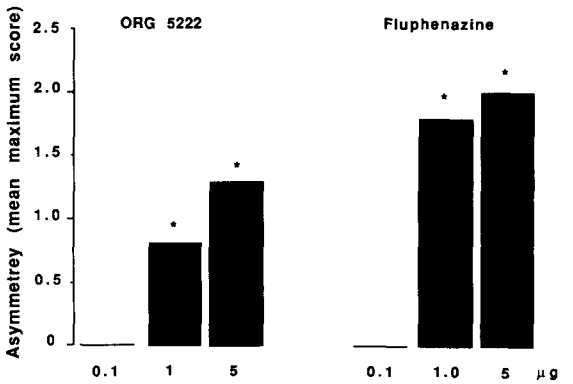


FIG. 3. Asymmetric body posturing in the rat following unilateral intrastriatal injections of fluphenazine and ORG 5222 (doses given in μg, vehicle injected into contralateral striatum) followed by subcutaneous challenge with apomorphine (0.25 mg/kg SC). Asymmetry (ipsilateral to the side of drug injection) was scored on a 0–3 system described in the Method section 15, 30 and 45 min after apomorphine administration and the maximal score recorded. Apomorphine failed to reveal any asymmetry in rats treated with unilateral intrastriatal injection of vehicle. Each value is the mean of 6 determinations where responses to fluphenazine and ORG 5222 (compared to vehicle injections) are significant to *$p<0.05$–$p<0.001$ (one-way ANOVA followed by Dunnett's t-test).

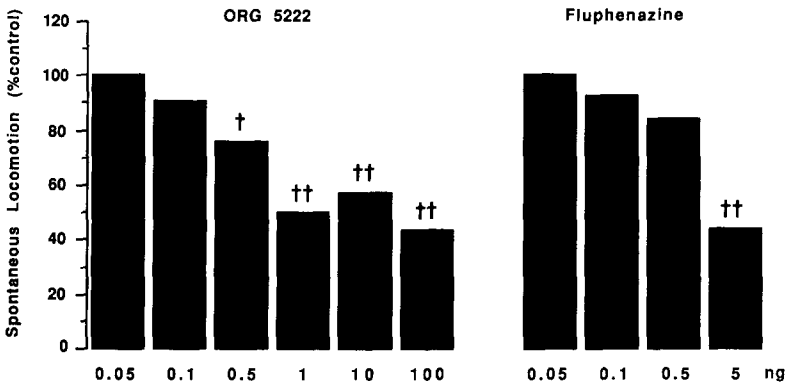


FIG. 4. Abilities of ORG 5222 and fluphenazine on bilateral injection into the nucleus accumbens of the rat to inhibit spontaneous locomotor activity. Hyperactivity was measured during the 30-min period following drug injection and is expressed as a % of the vehicle control values. n = 6. A significant reduction in spontaneous locomotor activity compared to control values is indicated as $\dagger p<0.05$ and $\dagger\dagger p<0.01$ (one-way ANOVA followed by Dunnett's *t*-test).

volume of 1 ml/kg (rat and marmoset) and 1 ml/100 g in the mouse.

RESULTS

The Cataleptic Ability of ORG 5222 and Fluphenazine in the Rat

Fluphenazine (0.5 to 4 mg/kg IP) induced a dose-related catalepsy over a narrow dose range and of maximum intensity. The onset of action was within 15 min using higher doses with a duration of action in excess of 4 hr. ORG 5222 was less potent than fluphenazine to induce catalepsy (ID_{50} values of 6.2 and 1.2 mg/kg respectively), and the distinguishing feature was the extended dose range required to demonstrate a dose-related effect (Fig. 2).

Asymmetric Body Posturing Following the Unilateral Intrastriatal Injection of ORG 5222 and Fluphenazine

The administration of apomorphine (0.25 mg/kg SC) to rats pretreated with a unilateral intrastriatal injection of ORG 5222 (0.1–5.0 μg) or fluphenazine (0.1–5.0 μg) (vehicle injections were made into the other striatum) caused an asymmetric body posturing ipsilateral to the side of the drug injection. A maximum response of score 2 was achieved 15 min after the injection of apomorphine in the fluphenazine-treated rats, although the response was not clearly dose related. Animals treated with ORG 5222 gave asymmetry scores ranging from zero to 2 with a mean score approximating to 1 (Fig. 3).

The Abilities of Intraaccumbens Injection of ORG 5222 and Fluphenazine to Inhibit Spontaneous and Amphetamine-Induced Activity in the Rat

A bilateral injection of ORG 5222 (0.1–1.0 ng) into the nucleus accumbens of the rat caused a dose-related decrease in spontaneous activity to 50% of control values. A one hundred-fold increase in dose failed to cause a further reduction in activity. The bilateral injection of fluphenazine (5 ng) reduced spontaneous activity to the same extent (Fig. 4).

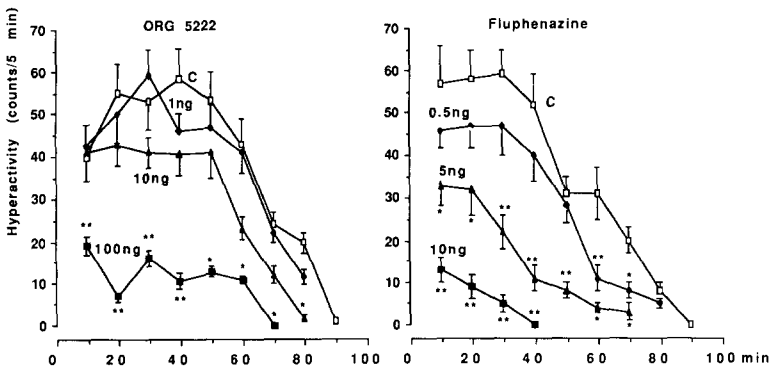


FIG. 5. Abilities of ORG 5222 and fluphenazine to inhibit the hyperactivity caused by the bilateral injection of amphetamine (10 μg) into the nucleus accumbens of the rat. The control response of animals receiving amphetamine plus vehicle is indicated C (□). Data are given for ORG 5222 or fluphenazine (closed symbols, nanogramme doses indicated) administered as 30-min pretreatment into the nucleus accumbens. n = 6. Vertical lines indicate s.e. means. Significant reductions in the amphetamine response are indicated as $*p<0.05$, $**p<0.01$ (two-way analysis of variance followed by Dunnett's *t*-test).

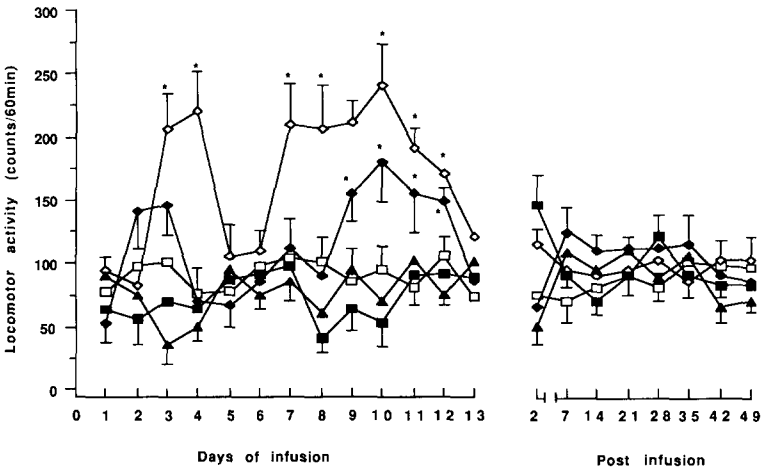


FIG. 6. The ability of ORG 5222 to modify the locomotor hyperactivity (measured in photocell cages and expressed as counts per 60 min) of rats during the 13 days of dopamine infusion (25 μg/24 hr, 0.48 μl/hr) into the nucleus accumbens and on days 2 to 49 after withdrawal of treatments. (◇) Indicates the responses of rats receiving intraaccumbens dopamine (plus vehicle for ORG 5222) and (□) the responses of rats receiving intraaccumbens vehicle for dopamine. The responses to dopamine combined with ORG 5222 0.025 (◆), 0.05 (▲) and 0.1 mg/kg (■) IP b.i.d. are shown. n=6. Vertical bars indicate s.e. means. Significant increases in locomotor activity to above vehicle control values are indicated as $*p<0.05$–$p<0.001$ (two-way analysis of variance followed by Dunnett's t-test).

The bilateral injection of amphetamine (10 μg) into the nucleus accumbens caused a peak increase in activity 20 to 40 min after injection, before declining to control values after 90 to 100 min. The bilateral intraaccumbens injection of fluphenazine (0.5–10 ng) administered as a 30-min pretreatment caused a dose-related inhibition of the amphetamine effect. Pretreatment with ORG 5222 at 1 ng failed to inhibit amphetamine-induced hyperactivity, 10 ng caused an inconsistent antagonism, whereas 100 ng markedly antagonised the amphetamine-induced effect (Fig. 5).

The Ability of ORG 5222 to Inhibit the Hyperactivity Caused by the Infusion of Dopamine Into the Nucleus Accumbens of the Rat

Locomotor activity in the rat during a 13-day intraaccumbens infusion of dopamine (25 μg/24 hr) peaked on days 4 and 10. A twice daily treatment with ORG 5222 (0.025 mg/kg IP) during the period of dopamine infusion reduced the hyperactivity response which was abolished using the higher doses of 0.05 and 0.1 mg/kg IP). Following cessation of the dopamine/ORG 5222 regimen, locomotor activity was maintained at preinfusion levels (Fig. 6).

The Effects of ORG 5222 and Fluphenazine on the Increased Responsiveness to the Locomotor Stimulant Effects of (−)NPA Caused by the Bilateral Infusion of Dopamine Into the Nucleus Accumbens of the Rat

Within 1 to 2 weeks after discontinuing a dopamine infusion, animals initially selected as 'low activity' responders to (−)NPA developed an approximately three-fold increase in responsiveness to the locomotor stimulant effects of (−)NPA. The enhanced responsiveness persisted for the 10-week duration of experiment. The administration of ORG 5222 (0.05 mg/kg IP b.i.d.) during the period of dopamine infusion attenuated the enhanced responsiveness to (−)NPA after cessation of treatment. Thus, the increased

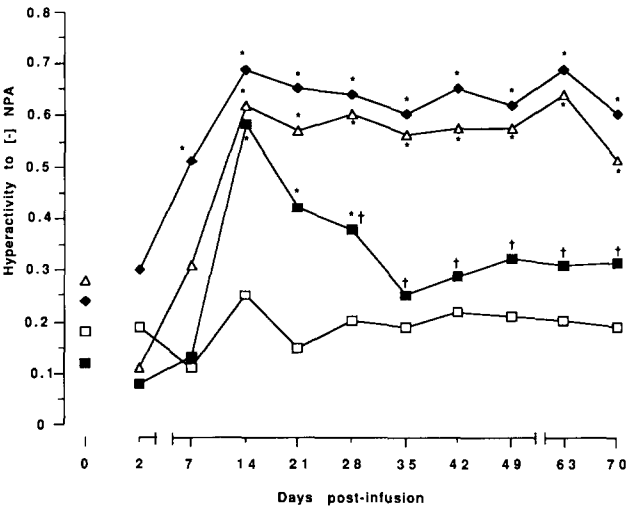


FIG. 7. The effects of ORG 5222 and fluphenazine to modify the changed responsiveness to the locomotor stimulant effects of (−)N-n-propyl norapomorphine [(−)NPA] caused by the bilateral infusion of dopamine into the nucleus accumbens of the rat. Animals were initially selected as 'low activity' responders to (−)NPA (0.05 mg/kg SC) (see text) and the initial locomotor activity count is indicated by the symbols on day 'zero.' The preselected animals received a 13-day infusion of vehicle (□), dopamine (25 μg/24 hr) (△) or dopamine plus fluphenazine 0.1 mg/kg IP b.i.d. (◆) or ORG 5222 (0.05 mg/kg IP b.i.d.) (■) and the responsiveness of such animals to (−)NPA (0.05 mg/kg SC) assessed on days 2 to 70 postdopamine infusion/drug treatment. Each value is the mean of 5 determinations where S.E.M.s were in the range 9–15%. The significance of the increase in responsiveness to (−)NPA compared to control values (□) is indicated $*p<0.001$; the significance of the prevention by ORG 5222 of the increased responsiveness to (−)NPA caused by the dopamine infusion is indicated $\dagger p<0.01$–0.001 (two-way ANOVA followed by Dunnett's t-test for multiple comparisons).

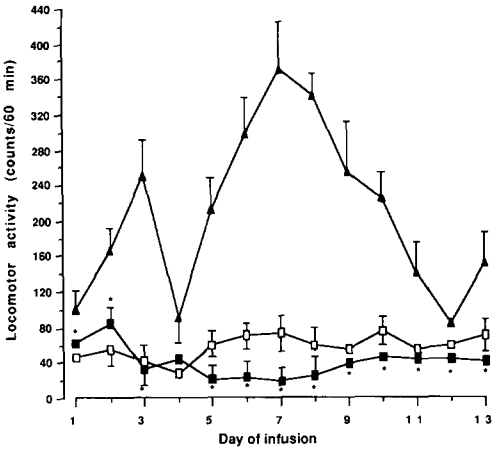


FIG. 8. The ability of ORG 5222 to antagonise the locomotor activity (measured in cages equipped with photocells and expressed as counts/60 min) of marmosets during the 13 days of dopamine infusion (25 μg/24 hr, 0.48 μl/hr) into the nucleus accumbens. (▲) Indicates the response of marmosets receiving intraaccumbens infusion of dopamine (plus vehicle for ORG 5222), (□) intraaccumbens infusion of vehicle for dopamine or (■) dopamine infusion plus ORG 5222 administered 0.025 mg/kg IP b.i.d. Vertical bars indicate s.e. means. Significant decreases in locomotor activity compared to dopamine control values are indicated *$p<0.05$–$p<0.01$ (two-way analysis of variance followed by Dunnett's *t*-test).

responsiveness to (−)NPA declined rapidly over a two- to three-week period, returning to values not significantly different from vehicle-treated controls. In contrast, fluphenazine (0.1 mg/kg IP b.i.d.) administered with the dopamine infusion failed to attenuate the increasing responsiveness to (−)NPA after cessation of fluphenazine/dopamine treatment (Fig. 7).

The Ability of ORG 5222 to Antagonise the Locomotor Hyperactivity Caused by the Infusion of Dopamine Into the Ventral Striatum of the Marmoset

The 13-day infusion of dopamine (25 μg/24 hr, 0.48 μl/hr) into the ventral striatum caused an approximate four-fold increase in locomotor activity. The increase in activity was not observed in animals that received a combined treatment with ORG 5222 (0.025 mg/kg IP b.i.d.) (Fig. 8).

The Effect of ORG 5222 and Fluphenazine to Modify the Reduced Responsiveness to (−)NPA Caused by the Bilateral Infusion of Dopamine Into the Ventral Striatum of the Marmoset

Within 5 days of discontinuing a 13-day infusion of dopamine (25 μg/24 hr) into the ventral striatum, marmosets initially preselected as 'high activity' responders to (−)NPA showed a reduced responsiveness to the locomotor stimulant effects. This persisted for the 15-day duration of the experiment. Animals that had been treated with a combination of ORG 5222 (0.025 mg/kg IP b.i.d.) and dopamine infusion showed a reduced responsiveness to (−)NPA on the first day following discontinuation of treatment. However, on the subsequent days, the responsiveness of the marmosets to (−)NPA was not significantly different from vehicle-treated controls. In contrast, the administration of fluphenazine (0.1 mg/kg IP b.i.d.) with dopamine failed to prevent the subsequent change in responsiveness to (−)NPA following cessation of treatment (Fig. 9).

Disinhibitory Effects of ORG 5222 and Diazepam in the Light/Dark Test in the Mouse

Control (vehicle)-treated mice, when placed into the brightly

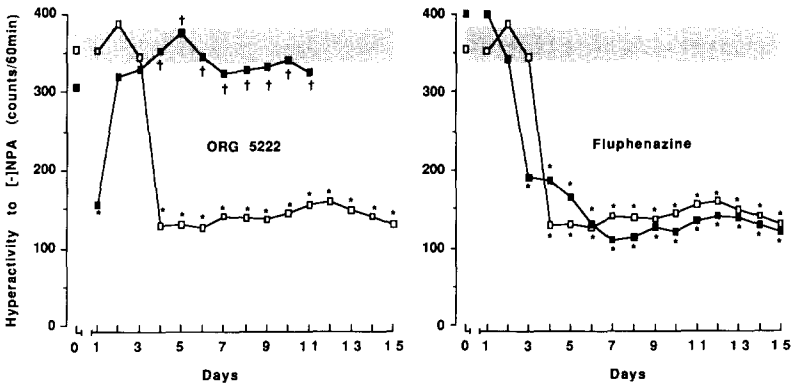


FIG. 9. The effect of ORG 5222 or fluphenazine to modify the changed responsiveness to the locomotor stimulant effects of (−)N-n-propyl norapomorphine [(−)NPA] caused by the bilateral infusion of dopamine into the ventral striatum of the marmoset. Animals were initially selected as 'high activity' responders to (−)NPA (0.05 mg/kg SC) (see text) and the initial locomotor activity count is indicated by the open symbols on day 'zero.' The preselected animals received a 13-day infusion of dopamine (25 μg/24 hr) (□), dopamine plus ORG 5222 (0.025 mg/kg IP b.i.d.) (■) or fluphenazine (0.1 mg/kg IP b.i.d.) (■) and the responsiveness of such animals to (−)NPA (0.05 mg/kg SC) assessed on days 1 to 15 postdopamine infusion/drug treatment. The hatched bands indicate the response to (−)NPA of marmosets that had received no infusion or drug treatments, i.e., vehicle controls. Each value is the mean of 4 determinations, S.E.M.s were in the range 8–16%. The significance of the reduction in responsiveness to (−)NPA compared to vehicle-treated controls is indicated *$p<0.001$; the significance of the prevention by ORG 5222 of the reducing responsiveness to (−)NPA is indicated †$p<0.001$ (two-way ANOVA followed by Dunnett's *t*-test for multiple comparisons).

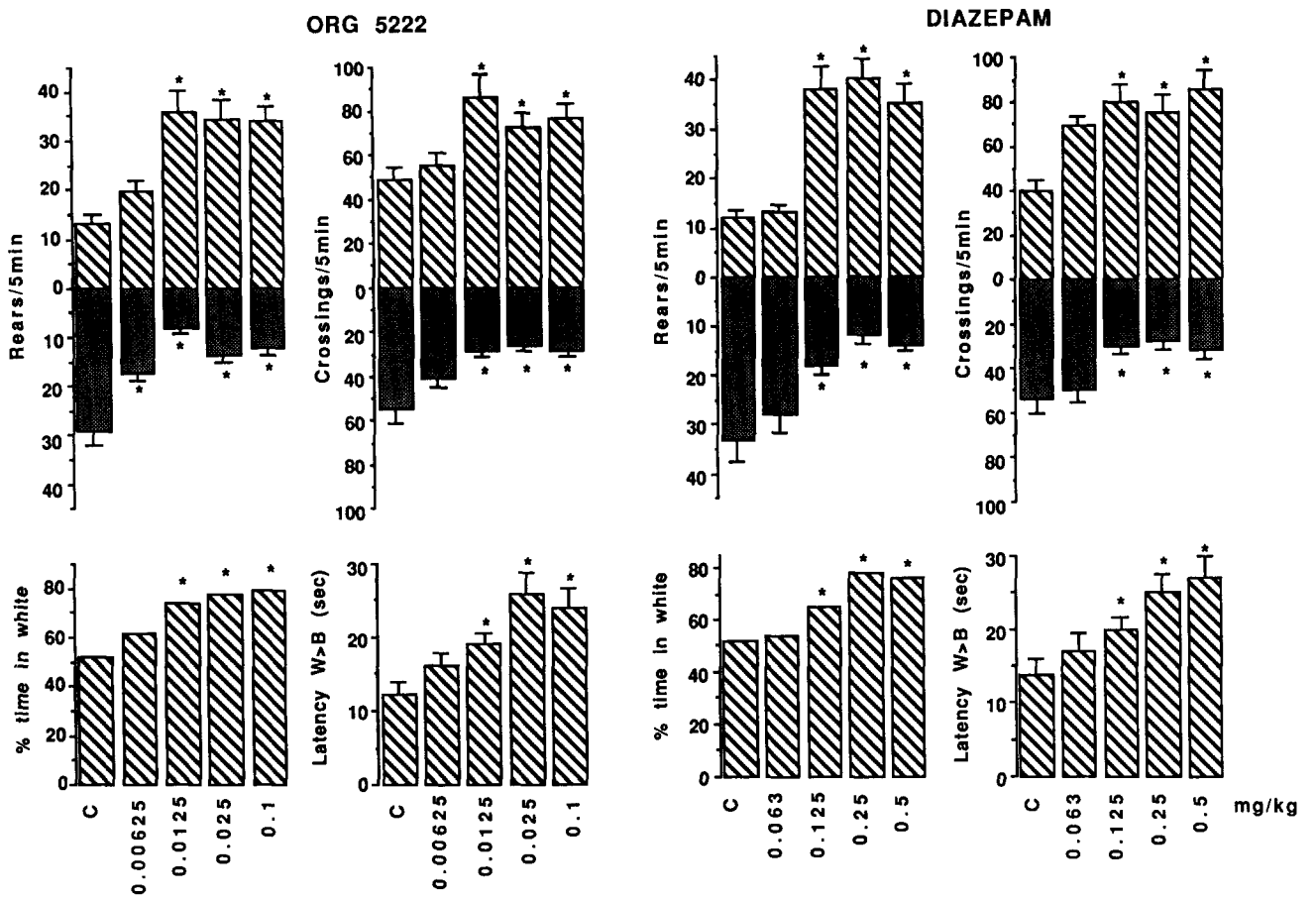


FIG. 10. Disinhibitory effects of ORG 5222 and diazepam in the light/dark discrimination test in the mouse. Mice were tested singly in an open-topped box three-fifths painted white and brightly illuminated (white section) and partitioned (with an interconnecting door) from the remainder of the box which was painted black and illuminated with red light (dark section). The floor of each section was lined into 9-cm squares. Changes in rearing (rears), line crossings (crossings), latency of movement from the white (W) to the black (B) section (after first placement into the white area) and % time spent in the white area were recorded. In the upper set of histograms, hatched columns indicate data for the light area, shaded columns for the dark area. Data obtained from control (C) and drug-treated mice were analysed using single-factor analysis of variance, and Dunnett's *t*-test. Significant increases/decreases in responding are indicated as $*p<0.05$–$p<0.001$. n = 5. Vertical bars indicate S.E.M.s. S.E.M.s were less than 11.1% on original data for calculations of % time in white section.

illuminated area of the two-compartment box, would move within approximately 12 sec into the dark environment, subsequently spending an approximately equal time in each area. Mice treated with ORG 5222 (0.0125–0.1 mg/kg IP) or diazepam (0.125–0.5 mg/kg IP) showed a preference for the brightly lit compartment, as indicated by an increased time spent, rearings and line crossings in the white area, and decreased in the black. In addition, mice treated with ORG 5222 showed an increased latency of initial movement from the white to the black section (Fig. 10).

The Effect of ORG 5222 and Diazepam in the Elevated Plus Maze Test in the Rat

The number of entries into and time spent in the furthermost sections of the open arms of the elevated plus maze was increased some 150–200% by ORG 5222 (0.025 and 0.1 mg/kg IP) (Fig. 11). At the doses used, ORG 5222 was as effective as diazepam (1.0 mg/kg IP) and approximately 10 times more potent. Vehicle-treated rats entered the furthermost sections of the closed arms on $16.1 \pm 2.1/19 \pm 1.2$ occasions (diazepam/ORG 5222) and drug treatments failed to significantly effect these values. Similarly, the time spent by vehicle-treated animals in the furthermost sections of the closed arms $19.7 \pm 3.5/26.7 \pm 4.6$ sec was not significantly modified by either ORG 5222 or diazepam.

DISCUSSION

ORG 5222 would appear less potent than fluphenazine to antagonise striatal function as revealed in both the catalepsy test and ability to induce asymmetric body posturing following unilateral intrastriatal injection in the rat. In these tests ORG 5222 was administered acutely, and the acute injection of ORG 5222 directly into the rat nucleus accumbens was again less potent than fluphenazine to antagonise the hyperactivity induced by amphetamine, although ORG 5222 and fluphenazine were equipotent to reduce spontaneous activity. Also, when administered peripherally, ORG 5222 and fluphenazine were equipotent to antagonise the hyperactivity induced by the continuous infusion of dopamine into the rat nucleus accumbens. The profiles of action of ORG 5222 in such tests is highly characteristic of a neuroleptic action in

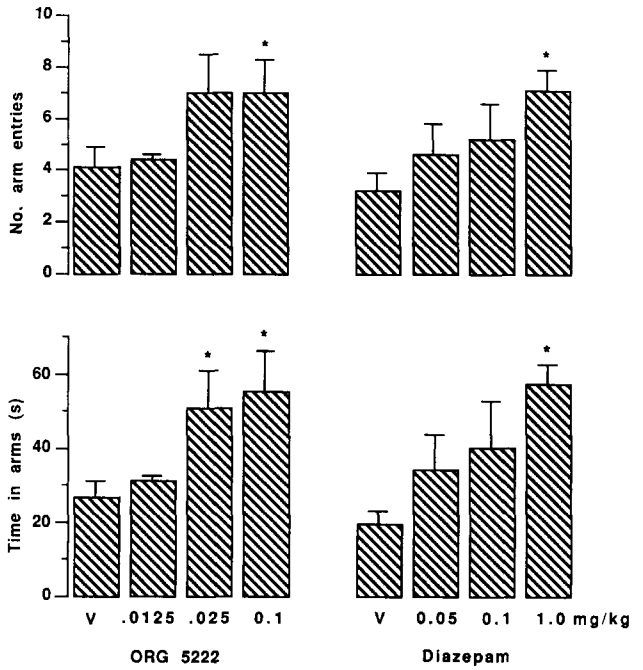


FIG. 11. The effects of ORG 5222 and diazepam on the behaviour of rats in the open arms of the elevated X-maze. Rats were treated IP with drug or vehicle (V) 45 min before testing for the number of entries and time spent in the open and closed arms of the X-maze during a 10-min test period. n=5–15. The significance of drug treatments compared to V is indicated *$p<0.05$ (one-way ANOVA followed by Dunnett's *t*-test).

the rodent, and the behavioural changes were achieved within a dose range known to modify dopamine turnover in the limbic and striatal areas of rat brain (16). Furthermore, the ability of ORG 5222 to antagonise locomotor activity induced by dopamine infusion into the ventral striatum of the marmoset is indicative of a similar action in the primate.

However, differences between ORG 5222 and fluphenazine were apparent on withdrawing ORG 5222/fluphenazine/dopamine infusion regimens. Thus, discontinuing a 13-day fluphenazine/dopamine infusion resulted in a rebound hyperactivity which was not observed following cessation of the ORG 5222/dopamine infusion regimen. The changes in spontaneous locomotion following the fluphenazine or haloperidol/dopamine treatments [see Costall and colleagues (6)] may be attributed to changes in dopamine receptor sensitivity (27), and do not appear to occur after withdrawal from treatment with low doses of ORG 5222 that inhibit the dopamine induced hyperactivity response. In future studies it would be of interest to determine whether this profile of action persists with the use of higher doses of ORG 5222.

Further evidence of differences between ORG 5222 and fluphenazine was apparent in their abilities to prevent changes in responsiveness to dopamine agonist challenge in both the rat and marmoset. Thus, rats preselected as 'low' and 'high' responders to the locomotor stimulant effects of (−)NPA show a reversal in responsiveness after dopamine infusion into the nucleus accumbens (5). In the present experiments rats preselected as 'low' activity responders showed, after discontinuing a dopamine infusion, an increasing responsiveness to the locomotor stimulant effects of (−)NAP, which was prevented by ORG 5222, but not fluphenazine administered during the dopamine infusion. A similar response was observed in the marmoset. Animals selected as highly responsive to the locomotor stimulant effects of (−)NPA showed a reducing sensitivity to (−)NPA after discontinuation of the dopamine infusion into the ventral striatum. The administration of ORG 5222 during the phase of DA infusion prevented such changes, whereas fluphenazine was ineffective. The mechanism of action of ORG 5222 to prevent such changes in responsiveness to dopamine agonist challenge is not known, but may reflect the affinity of ORG 5222 for neurotransmitter receptors in addition to dopamine, for example α_1-adrenoceptors and 5-HT_2 receptors (16), which may contribute to the regulation of limbic function. These possibilities can be further investigated using agents with a selectivity of action for the different receptor types, although it remains possible that the effects of ORG 5222 are due to an ability to block more than one receptor system. The failure of fluphenazine to prevent the changes in responsiveness to (−)NPA indicates that a dopamine receptor antagonism alone is unlikely to account for the effects of ORG 5222. Many of the extrapyramidal side effects of existing neuroleptic treatment appear related to an unrequired blockade of striatal dopamine receptors and the subsequent development of changes in dopamine receptor sensitivity (25). It is, therefore, an interesting finding that ORG 5222 can control a dopamine induced hyperactivity without causing a change in responsiveness to dopamine agonist challenge.

ORG 5222 was also distinguished from the classical neuroleptic agents such as fluphenazine and haloperidol by its ability to release behaviour in the mouse suppressed by bright illumination. In the two-compartment black and white test box, mouse aversion to the brightly illuminated white area is reduced by anxiolytic agents such as diazepam and buspirone and intensified by the anxiogenic agent FG7142 (10, 12, 15). Other psychopharmacological agents fail to induce specific changes in exploration in the test box and, in particular, classical neuroleptic agents cause a nonspecific reduction in behaviour in both the black and the white areas (10,15). Therefore, the ability of ORG 5222 to increase the time spent by mice in exploration of the white section and decrease such activities in the black presents as a profile of anxiolytic action. This was confirmed in the elevated plus maze test in the rat, where ORG 5222 enhanced the number of entries into and time spent in the furthermost sections of the open arms. Similarly to the mouse test, ORG 5222 was approximately ten times more potent than diazepam. The potency of ORG 5222 to release suppressed behaviour was threshold or similar to those doses inhibiting the consequences of a mesolimbic dopamine infusion, but one hundred times less than required to induce catalepsy.

Although it is unlikely that a dopamine receptor blockade contributes to the actions of ORG 5222 in reducing aversive behaviour, ORG 5222 has affinity for the 5-HT_3 receptor in radioligand binding assays (Barnes personal communication; pK_i 6.2 to displace [^{3}H]zacopride), and ondansetron and other 5-HT_3 receptor antagonists are very potent to release suppressed behaviour (23). Whilst a pK_i value of 6.2 is a thousand-fold less than recorded using ondansetron, ORG 5222 was also approximately a thousand-fold less potent in the behavioural tests. Therefore, it is possible that the modest affinity of ORG 5222 for 5-HT_3 receptors may contribute to its actions to reduce aversive responding, and also to reduce the consequences of a raised limbic dopamine function (8). Yet the affinity of ORG 5222 for 5-HT_3 receptors remains some thousand times less than the affinity for 5-HT_2 receptors (16), and it is perhaps a combination of effects on the 5-HT_2 and 5-HT_3 receptor that facilitate the release of suppressed behaviour (10,19).

In summary, ORG 5222 has an ability to antagonise a mesolimbic dopamine function and an anxiolytic profile of action in mouse and rat models. Such spectra of actions distinguish ORG 5222 from the classical neuroleptic agents and may reflect the broad profile of affinities for various neurotransmitter sites and particularly a dopamine/5-HT receptor blockade.

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