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Behavioural interactions between 5-hydroxytryptophan, neuroleptic agents and 5-HT receptor antagonists in modifying rodent responding to aversive situations

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1 The ability of 5-hydroxytryptophan, 5-HT_2 receptor antagonists and typical and atypical neuroleptic agents to modify behavioural responding to aversive situations was investigated in the mouse light/dark test and rat social interaction.

2 The administration of 5-hydroxytryptophan inhibited rat social interaction and the exploratory behaviour of mice in the light/dark test.

3 The 5-HT_2 receptor antagonists, ketanserin, ritanserin, MDL11939, methysergide and RP62203, the neuroleptic agents, spiperone, haloperidol and benperidol, and the atypical neuroleptic agent, clozapine, when administered alone failed to modify mouse or rat behaviour. In contrast, when administered alone, sulpiride in rats and mice and thioridazine in rats disinhibited behaviour.

4 Methysergide, RP62203, ketanserin, ritanserin and MDL11939 antagonized the inhibitory effects of 5-hydroxytryptophan or reversed the inhibitory effects to one of disinhibition.

5 Low doses of spiperone (but not haloperidol or benperidol) also antagonized the inhibitory effects of 5-hydroxytryptophan in the rat but not the mouse. Higher doses of the three neuroleptic agents caused locomotor depression in both rats and mice which obscured any specific changes in behavioural responding to the aversive situations.

6 The disinhibitory profile of sulpiride in both mice and rats and thioridazine in rats was evident during their interaction with 5-hydroxytryptophan. Thioridazine in the mouse and clozapine in rats and mice also reversed the inhibitory effects of 5-hydroxytryptophan to one of disinhibition.

7 In summary, we present evidence that the atypical neuroleptic agents, thioridazine and clozapine, with their known affinity for the 5-HT_2 receptors, can mimic the actions of reference 5-HT_2 receptor antagonists to antagonize the inhibitory effects of 5-hydroxytryptophan in rodent models of anxiety. The results are intepreted in terms of drug action on different 5-HT_2 and other 5-HT receptor subtypes. In addition, thioridazine and sulpiride have disinhibitory effects in their own right which remain to be explained.

Keywords: 5-Hydroxytryptophan; 5-HT_2 receptor antagonists; neuroleptics; clozapine; thioridazine; sulpiride; rodent behavioural responding; aversive situations

Introduction

The dopamine receptor antagonists have a long established role as the most effective treatment for the control of agitated and positive psychotic behaviour in schizophrenia. Yet some 10-20% of patients derive little benefit from typical neuroleptic drug therapy (Kanes, *et al.*, 1988) and in the neuroleptic-nonresponsive patient, the failure to treat the negative symptomatology has caused widespread concern (see Carpenter *et al.*, 1991). Whilst the negative symptoms remain the most debilitating component of chronic schizophrenia, either as a primary and discrete component or secondary to other factors (Carpenter & Conley, 1991), their underlying psychopathology is not understood. Therefore it has been inevitable that novel treatment strategies for this component of the illness have been based on empirical clinical findings.

One of the first observations was that pipamperone, a weak neuroleptic but potent 5-HT_2 antagonist, was reported to be helpful in chronic schizophrenia being disinhibitory, anti-autistic and facilitating re-socialisation (see Niemegeers, 1989). The relative importance of the 5-HT_2 receptor blockade was investigated with the selective 5-HT_2 receptor antagonist, ritanserin. In schizophrenia and also non-psychotic disorders, ritanserin caused an improvement in mood, an increase in slow wave sleep and a decrease in the extrapyramidal side effects of concurrent neuroleptic therapy (Reyntjens *et al.*, 1986; Janssen, 1987; Pangalila-Ratu Langi & Janssen, 1988). Subsequently, risperidone and other agents were synthesized which retained a high affinity for both dopamine and 5-HT_2 receptors (Janssen *et al.*, 1988), to ensure an efficacious treatment of both the positive and negative symptoms, with beneficial effects on mood and anxiety, and little extrapyramidal disturbance (Castelao *et al.*, 1989; Messoten *et al.*, 1989; Meco *et al.*, 1989; Leysen *et al.*, 1993).

A second important advance in the treatment of the negative symptoms has come from the use of clozapine, initially introduced as an antipsychotic agent with a very low incidence of extrapyramidal side effects (Angst *et al.*, 1971). Clozapine was reported to be superior to chlorpromazine in treating the negative symptoms of schizophrenia (Fischer-Cornelssen & Ferner, 1976; Claghorn *et al.*, 1987; Kane *et al.*, 1988; 1990) and is becoming a most significant therapy for treatment-resistant schizophrenia (Carpenter & Conley, 1991). Meltzer (1989, 1991) has proposed that the antagonistic effects of clozapine at the 5-HT_2 receptor are important for its use in schizophrenia and may reflect a dysfunction in both the 5-HT and dopamine systems.

The actions of clozapine and other 5-HT_2/dopamine receptor antagonists in treating the negative symptoms of social isolation, flat or inappropriate affect, poverty of speech and

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lack of personal care may be perceived as a disinhibitory effect, facilitating re-socialisation and reducing anxiety and inappropriate affect. Indeed, such properties have been described for atypical and even typical neuroleptic agents in non-psychotic illness (Robertson & Trimble, 1982; Johnson, 1983; Standish-Barry *et al.*, 1983). A better understanding of the pharmacological basis of the efficacy of clozapine and other agents in ameliorating negative symptoms would aid the development of more effective treatments. But such an understanding has been delayed by the absence of a correlate to such behaviours in animals.

Recently it has been reported that ritanserin and other 5-HT_2 receptor antagonists disinhibit behavioural responding to aversive situations and attenuate the inhibitory effects induced by 5-hydroxytryptophan and 5-HT agonists in rodents (Kennett, 1992; Kennett *et al.*, 1989; 1994a; Costall *et al.*, 1993a, b; Cheng *et al.*, 1994). If these interactions could also be shown to occur using neuroleptic agents with 5-HT receptor antagonist properties, it might provide a pharmacological basis for an improved understanding of their disinhibitory effects in man. With this in mind, the aim of the present study was two fold. Firstly, to attempt a further characterization of the 5-HT_2 receptor(s) mediating the inhibitory effects of 5-hydroxytryptophan using 5-HT receptor antagonists with predominant but differing affinities for the 5-$HT_{2A/2C}$ receptors; secondly, to determine whether the profile of action of such compounds can be detected in typical and atypical neuroleptic agents.

Methods

Animals

Male albino BKW mice (Bradford strain) were used throughout the studies. The mice, weighing 30–35 g, were housed in groups of 10 in conditions of constant temperature ($21 \pm 1°C$) and controlled lighting (dark period 07 h 00 min–19h 00min).

Male Lister Hooded rats (250–300 g) (University of Bradford bred) were housed in groups of five and kept in conditions of standard temperature ($21 \pm 1°C$) on a 12 h light/dark cycle with lights off at 19 h 00 min.

Both mice and rats were given free access to water and fed *ad libitum* on a CRM high protein diet obtained from Special Diet Services.

The cleaning and feeding of animals was performed at fixed periods by specified animal husbandry staff.

Behavioural testing of mice

Mice were removed from the dark holding rooms and placed into a dark container for transportation using constant removal conditions to a dark test room, designated exclusively for mouse behavioural testing and for specific personnel. Tests for changes in behaviour were conducted between 08h 00min and 13h 00min in the darkened room illuminated with a red light. After a 1 h period of adaptation to the new environment, mice were placed into the light compartment of the light/dark test box which was located in an ante-room on a bench 1 meter above floor level. The box ($45 \times 27 \times 27$ cm high) was open-topped and the base lined into 9 cm squares, two-fifths painted black and illuminated by red light (1×60 W, 0 Lux) and partitioned from the remainder of the box which was painted white and brightly illuminated with a 1×60 W (400 Lux) light source, the red and white lights being located 17 cm above the box. The compartments were connected by an opening, 7.5×7.5 cm, located at floor level in the centre of the partition. Mice received drug or vehicle and after 40 min were placed in the centre of the white, brightly lit area and the operator withdrew from the ante-room. The mice were observed by remote video recording and four behaviours were noted (a) the number of exploratory rearings in the light and dark sections, (b) the number of line crossings in the light and dark sections, (c) the time spent in the light and dark areas and (d) the latency of the initial movement from the light to the dark area.

Behavioural testing of rats

Tests were conducted between 13h 00min and 18h00min in an illuminated room using a social interaction test based on the model of File (1980). The apparatus used for the detection of changes in social interaction and exploratory behaviour in rats consisted of an open-topped box (51×51 cm and 20 cm high) with 17×17 cm areas marked on the floor. Two naive rats, from separate housing cages, received an injection of drug or vehicle (40 min pretreatment) and were placed in the brightly illuminated area of the test box and their behaviour observed over a 10 min period by remote video recording. Two behaviours were noted: (1) social interaction between the animals was determined by timing (s), sniffing of partner, crawling under or climbing over partner, genital investigation of partner, following partner and (2) exploratory locomotion was measured as the number of crossings of the lines marked on the floor of the test box.

Experimental design

Mice and rats were used once only. The dose-related effects of 5-hydroxytryptophan (5-HTP) were investigated using vehicle and 4 treatments of 5-HTP, 5 (mice) or 6 (rats) animals per treatment, the experiments being repeated once. Preliminary experiments using animals in treatment groups of 5 or 6 also established the dosage regimens that were required to reveal the effects of the interaction between 5-HTP and a potential antagonist. Subsequently, four regimens were used in the experiments designed to investigate the interactions between 5-hydroxytryptamine and dopamine receptor antagonists with 5-HTP; (a) vehicle + vehicle, (b) 5-HTP + vehicle, (c) drug and vehicle and (d) 5-HTP + drug. The total number of treatments involved in any one 5-HTP/drug interaction varied from 10 to 16 (see Figures 1 and 2), necessitating a number of sessions, 5 in the mouse and 6 in the rat on different days to generate final group sizes per treatment of 10 (mouse) and 12 (rat). All treatment groups were equally represented on each day of testing and data were initially analysed by two factor analysis of variance (Statview 4.02). This allowed for testing of the factors of each treatment (including the vehicle and 5-HTP 'controls') and session; pertinent F values are shown in the results section. This initial analysis ensured that firstly, the vehicle control responses, the 5-HTP response and the effect of drug treatment from day to day could be shown to be statistically indistinguishable, since variation in the basal level of responding can notably influence drug action in rodent models of anxiety (File *et al.*, 1992). Secondly, it ensured a comparability of treatments between sessions before collapsing the data for final analysis. When a significant overall F ratio was detected, pairwise comparisons of treatment groups were undertaken using a single factor analysis of variance followed by Dunnett's procedure for comparing treatments with control. Data are expressed as mean ± s.e.mean.

A variation in response was minimised by closely controlled in-house breeding of animals, consistent procedures and personnel for animal husbandry and transportation and handling of animals, performance of experiments in dedicated laboratories and use of staff trained to ensure a constant level of experimental skill in the handling, injection and use of animals. With such factors in mind the effect of 'session' did not achieve significance and no data from any experiments were required to be discarded.

Drugs

5-Hydroxytryptophan methyl ester (Sigma), clozapine and methysergide maleate (Sandoz) were sonicated and dissolved

Table 1 Effects of 5-hydroxytryptophan (5-HTP, 6.25 to 50 mg kg^{-1}) in increasing mouse behaviour in the dark compartment of the light/dark test box and in reducing social interaction in the rat

	Mouse light/dark test		*Rat*	
Treatment (mg kg^{-1})	*Latency light to dark* (s)	*% Time in dark*	*Social interaction* (s)	*Line crossings/ 10 min*
Vehicle	10.5 ± 0.6	46.8 ± 4.62	57.5 ± 6.1	118 ± 14
5-HTP				
6.25	11.3 ± 0.9	58.0 ± 7.3	50.7 ± 6.3	125 ± 11
12.50	10.0 ± 0.5	52.6 ± 5.3	52.9 ± 7.1	106 ± 16
25.00	5.6 ± 0.3*	68.6 ± 5.5	24.7 ± 3.3*	117 ± 13
50.00	1.7 ± 0.2*	77.6 ± 8.6*	17.9 ± 3.8*	120 ± 20

In the mouse, the latency of first movement from the light to the dark compartment and % time spent in the dark area over a 5 min period are shown. In the rat, the time spent in social interaction and line crossings during a 10 min period is presented. Values represent the mean ± s.e.mean of $n = 10$ (mice) and $n = 12$ (6 pairs, rat). Significant increases or decreases in response compared to vehicle are indicated $*P < 0.005$ (one-way ANOVA followed by Dunnett's t test).

in distilled water, ritanserin hydrochloride and ketanserin hydrochloride (Janssen) were prepared in a 10% solution of polyethylene glycol and dilutions made with saline, haloperidol was prepared from Seranace injection in saline, thioridazine hydrochloride (Sandoz), sulpiride hydrochloride (Delagrange), benperidol and spiperone (Janssen), RP62203((2-[3-(4-(4-fluorophenyl)-piperazinyl)propyl]naphto [1,8-cd]isothiazole-1,1-dioxide)) (Rhône-Poulenc Rorer) and MDL11939 ((±)α-phenyl-1-(2-phenylethyl)-4-piperidinemethanol; Marion Merrell Dow) were dissolved in the minimum amount of acetic acid and distilled water, dilutions being prepared with saline. Drugs were injected intraperitoneally in a volume of 1 ml kg^{-1} in the rat and 1 ml 100 g^{-1} in the mouse, doses being expressed as the base.

Results

General observations

The nature and illumination of the test box ensured that when vehicle-treated control mice were placed into the light compartment of the test box they moved within approximately 10 to 12 s into the dark compartment, subsequently spending approximately 50% of their time in either compartment. The aversive nature of the light compartment caused a reduced level of rearings and line crossings in this as compared to the dark compartment. Typically, mice showed rearings in the light/dark of 20/40 and 50/70 per 5 min and line crossings of 25/35 and 45/65 per 5 min respectively (see Figures 7 and 8).

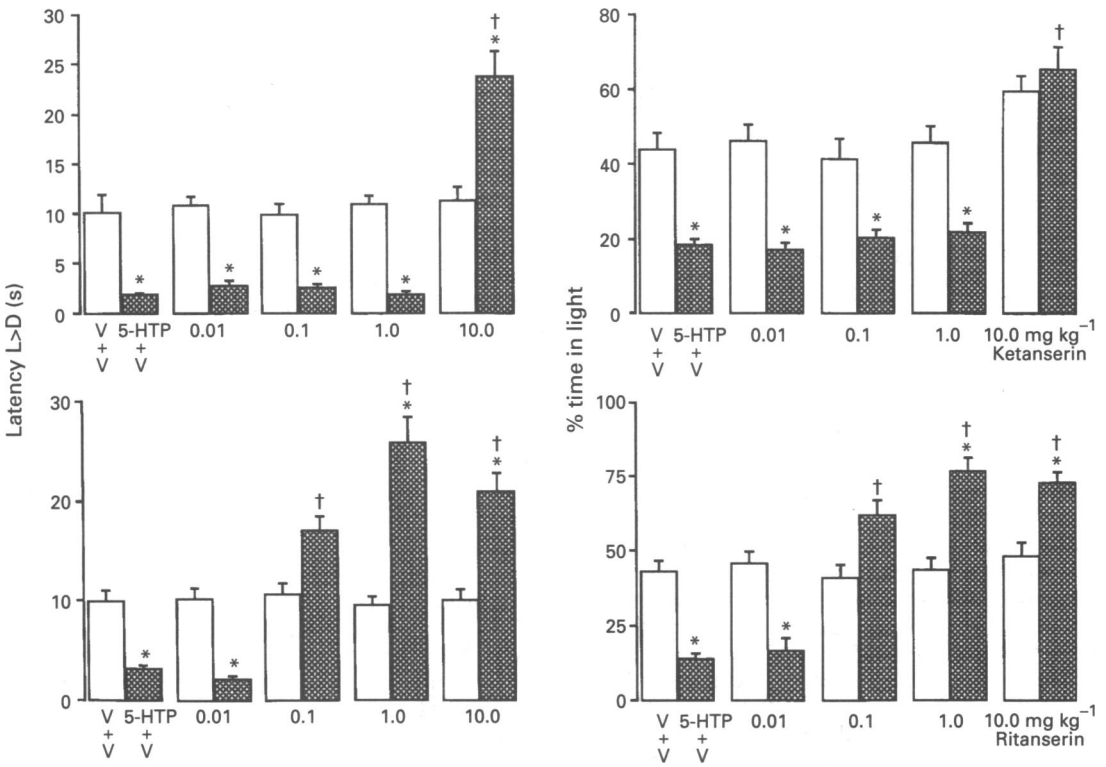

Figure 1 The effect of ritanserin and ketanserin in the light/dark exploration test in the mouse. Animals received an intraperitoneal injection of vehicle (V, open columns) or 5-hydroxytryptophan (5-HTP, 50 mg kg^{-1}, cross-hatched columns) plus vehicle or ritanserin or kitanserin (0.01 – 10 mg kg^{-1}) with testing 40 min after the last treatment. Latency of first movement from the light (L) to the dark (D) compartment and % time spent in the light compartment during the 5 min test period are shown. $n = 10$, mean values ± s.e. mean are shown $*P < 0.05$ compared to control V + V and $\dagger P < 0.05$ compared 5-HTP + V; (ANOVA and Dunnett's t test).

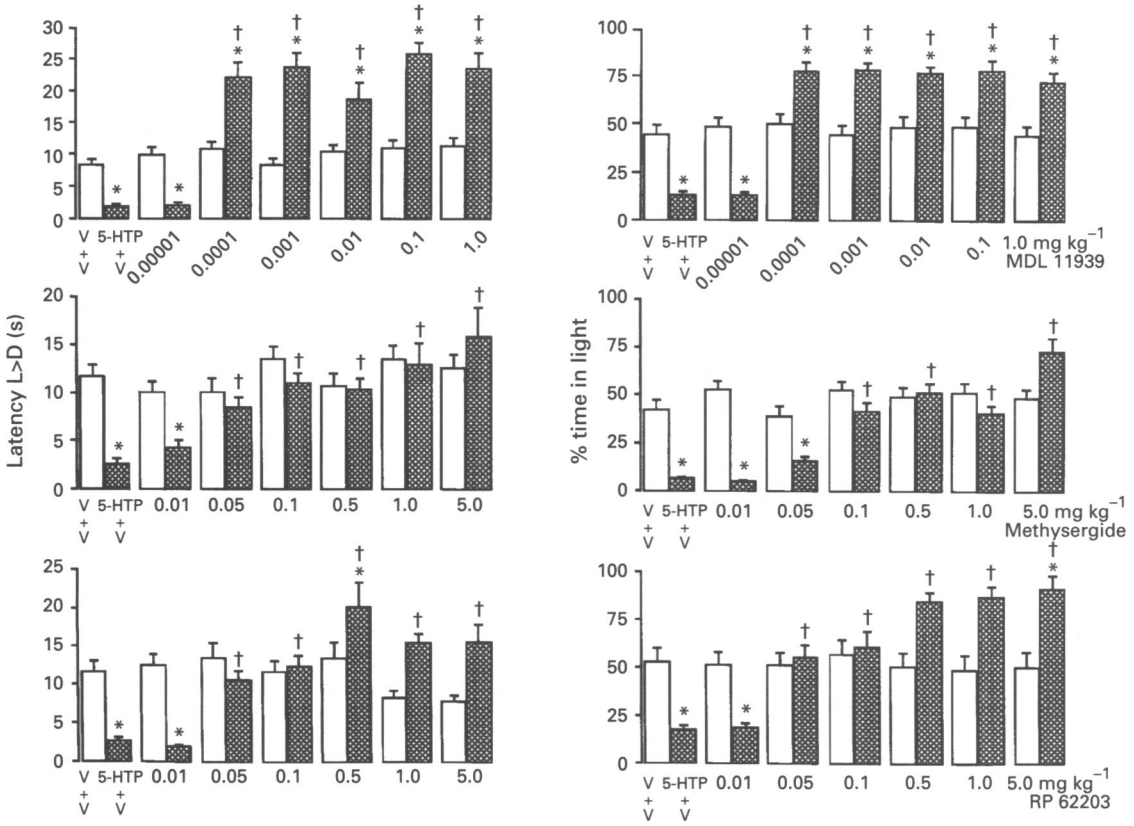

Figure 2 The effect of MDL11939, methysergide and RP62203 in the light/dark exploration test in the mouse. Animals received an intraperitoneal injection of vehicle (V, open columns) or 5-hydroxytryptophan (5-HTP, 50 mg kg^{-1}, cross-hatched columns) plus vehicle or MDL11939, methysergide or RP62203 at the doses indicated with testing 40 min after the last treatment. Latency of first movement from the light (L) to the dark (D) compartment and % time spent in the light compartment during the 5 min test period are shown. $n=10$, mean values ± s.e. mean are shown. *$P<0.05$ compared to control V + V and †$P<0.05$ compared to 5-HTP + V; (ANOVA and Dunnett's t test).

Drugs causing a selective increase in the time spent in one compartment were associated with increases in rearings and line crossings in that compartment and decreases in the other. Therefore the presentation of data is usually restricted to the values for latency of first movement to the dark compartment and the % time spent in the light compartment. Non-selective changes in rearings and line crossings reflect a non-specific change in motor performance and are particularly important in an interpretation of neuroleptic induced effects: such data are included where appropriate.

Vehicle-treated control rats under conditions of unfamiliarity and high illumination spent 40 to 60 s in social interaction within the 10 min period. Drug-induced increases or decreases in social interaction were not associated with any changes in locomotor activity (line crossings) unless such data are presented.

The effect of 5-hydroxytryptophan (5-HTP)

Mice were used in treatment (vehicle and 5-HTP) (6.25–50 mg kg^{-1}) groups of 5 in two sessions; ANOVA indicated in both sessions a highly significant interaction for latency of first movement from the light to the dark compartment ($F(4,45)=71.33$, $P<0.0001$ and $F(4.45)=9.41$, $P<0.0001$) and for the % time spent in the dark area of the test box ($F(4,45)=3.72$, $P<0.05$ and $F(4,45)=4.83$, $P<0.005$). There was no significant variation in vehicle or 5-HTP treatment response between the two sessions ($P>0.05$) and the data between the sessions was collapsed for the presentation of data in Table 1. 5-HTP caused a dose-related decrease in the latency of first movement from the light to the dark compartment, achieving significance at 25.0 and 50 mg kg^{-1} ($P<0.001$). There was also a trend for mice to spend more time in the dark area and this achieved significance at the dose of 50 mg kg^{-1} ($P<0.005$). Changes in rearings and line crossings followed the changes in time spent in the two compartments of the test box.

Rats were used in treatment (vehicle and 5-HTP 6.25–50 mg kg^{-1}) groups of 6 in two sessions; ANOVA indicated in both sessions a highly significant interaction $F(4,55))=11.74$, $P<0.0001$ and $F(4,55)=13.5$, $P<0.0001$) for social interaction, with no significant F ratios (0.22 and 0.49 $P>0.8$) for line crossing values. There was no significant variation in vehicle or 5-HTP-induced effects between sessions ($P>0.05$). The social interaction and line crossing data were collapsed across sessions, 5-HTP causing a dose-related reduction in social interaction to a maximum of 68%, achieving significance at doses of 25 and 50 mg kg^{-1} (Table 1).

The effect of ritanserin and other 5-HT$_2$ receptor antagonists

An investigation of the interaction between 5-HTP and 5-HT$_2$ receptor antagonists involved the use of up to 16 treatments including 5-HTP and vehicle-treated controls and 5 (mouse) or 6 (rat) test sessions. Within an experiment the effect of session for vehicle, 5-HTP, 5-HT$_2$ receptor antagonist or 5-HTP/5-HT$_2$ receptor antagonist interaction was never significant, with ANOVA F ratios giving values where $P>0.05$.

In the mouse light/dark test, the ability of ritanserin to antagonize the inhibitory effect of 5-HTP (Costall *et al.*, 1993a) was confirmed and extended to ketanserin, RP62203, methysergide and MDL11939. Vehicle control responses remained consistent throughout the 5 experiments, with values of latency of first movement from the light to the dark compartment being in the range 10.34 ± 0.64 s. The effect of 5-HTP (50 mg kg^{-1}) also remained highly reproducible, reducing the latency values by 76.8 ± 2.13% throughout the 5 experiments. Similarly, in these 5 experiments the vehicle-treated control

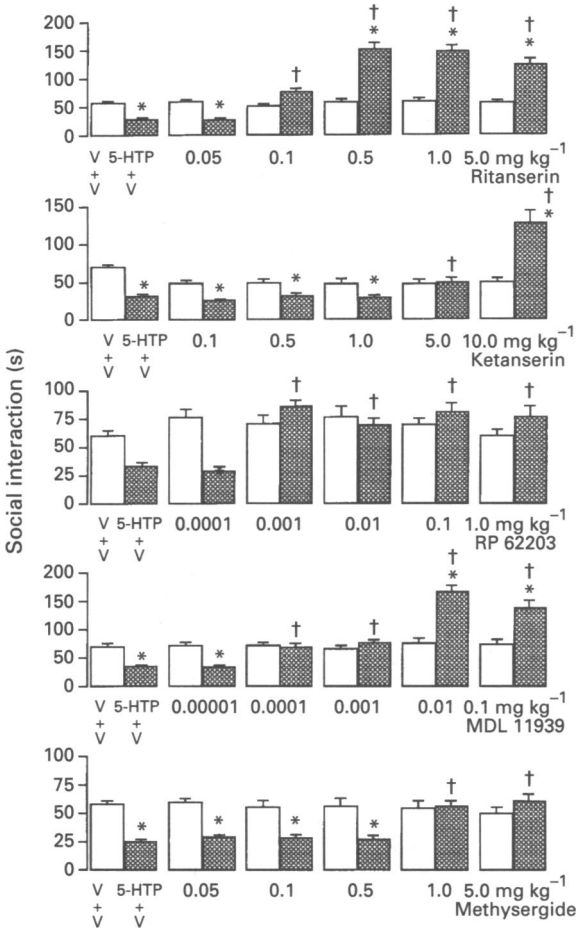

Figure 3 The effect of ritanserin and the other 5-HT_2 receptor antagonists on social interaction in rats. Pairs of rats received an intraperitoneal injection of vehicle (V, open columns) or 5-hydroxytryptophan (5-HTP, 50 mg kg^{-1}, cross-hatched columns) plus vehicle or ritanserin, ketanserin, RP62203, MDL11939 and methysergide at the doses indicated with testing for 10 min, 40 min after the last treatment. $n=6$ pairs per group, mean values ± s.e. mean are shown. *$P<0.05$ compared to V+V; †$P<0.05$ compared to 5-HTP+V; (ANOVA and Dunnett's t test).

mice spent 43.3±2.51% of their time in the light area, the 5-HTP treatment consistently reducing the time spent in the light area by 69.5±4.12%. The effect of 5-HTP in reducing the latency of first movement from the light to the dark compartment and the % time spent in the light area was antagonized by ritanserin, ketanserin, RP62203, methysergide and MDL11939, but with notable differences in potencies.

MDL11939 was the most potent compound tested, antagonizing the effects of 5-HTP at a dose of 0.0001 mg kg^{-1}. MDL11939 was five hundred times more potent than RP62203, one thousand times more potent than methysergide and ritanserin and one hundred thousand times more potent than ketanserin. There were also qualitative differences in the antagonisms afforded by the five agents. Thus ketanserin, ritanserin and MDL11939 not merely antagonized the inhibitory effects of 5-HTP but reversed the behaviour to one of disinhibition i.e. the effect of the 5-HTP/5-HT_2 receptor antagonist interaction was to increase values to above those of the vehicle-treated controls. However, with methysergide at doses fifty times that causing an antagonism of the inhibitory effects of 5-HTP, there was no significant reversal of the effects of 5-HTP. Furthermore, whilst there was a clear trend for RP62203 to reveal a disinhibitory potential of 5-HTP at doses higher than required to antagonize the effects of 5-HTP, the disinhibitory potential failed to achieve a consistently significant response. Notwithstanding the use of extensive dose-ranges of the 5-HT receptor antagonists, none of the 5 agents when administered alone modified mouse behaviour as compared to the vehicle treated controls (Figures 1 and 2).

In the five experiments investigating the interaction between 5-HTP and the 5-HT_2 receptor antagonists to modify rat social interaction, vehicle control responses were in the range of 62.9±2.93 (s), remaining constant throughout the experiments. Also 5-HTP (50 mg kg^{-1}) reliably reduced social interaction between experiments by 47–50%.

The five 5-HT receptor antagonists when administered alone failed to modify rat social interaction. The inhibitory effect of 5-HTP in reducing social interaction was antagonized by MDL11939, ritanserin and ketanserin which were effective in doses as low as 0.0001, 0.1 and 5.0 mg kg^{-1} respectively, doses directly comparable to those antagonizing the effects of 5-HTP in the mouse. However, RP62203 (at 0.001 mg kg^{-1}) and methysergide (at 1.0 mg kg^{-1}) were fifty times more and ten times less potent respectively in antagonizing the inhibitory effects of 5-HTP in the rat as compared to the mouse. As previously recorded in the mouse experiments, the antagonism of the effects of 5-HTP in the rat using ritanserin, ketanserin and MDL1939 was associated with a reversal of the effects of 5-HTP to one of disinhibition, i.e. an increase in social interaction to values above vehicle treated controls. The antagonism afforded by methysergide and RP62203 was not associated with a reversal of the effects of 5-HTP, values returning to those of the vehicle-treated controls (Figure 3).

The effect of the atypical neuroleptic agents, thioridazine, clozapine and sulpiride

In the three experiments investigating the interaction between 5-HTP and thioridazine, clozapine or sulpiride, the basal responding of the mice and rats to vehicle or to the inhibitory effects of 5-HTP was indistinguishable from that reported in the above experiments. Within an experiment, the effect of session for vehicle, 5-HTP, thioridazine, clozapine, sulpiride or the interaction between 5-HTP and the latter three compounds, was never significant, with ANOVA F ratios giving values where $P>0.05$.

Thioridazine (0.1–5.0 mg kg^{-1}) and clozapine (0.05–5.0 mg kg^{-1}) when administered alone failed to modify mouse behaviour in the light/dark test. Thioridazine at 1.0 and 5.0 mg kg^{-1} antagonized the inhibitory effects of 5-HTP, reversing the behaviour from a decreased to an increased exploration of the light compartment. Clozapine at 0.5 mg kg^{-1} also antagonized the inhibitory effects of 5-HTP and at 1.0 mg kg^{-1} reversed the effects, mice showing the delayed latency of first movement from the light to the dark compartment and increasing the time spent in the light area. At a higher dose of clozapine 5.0 mg kg^{-1}, the antagonism of the inhibitory effects of 5-HTP failed to achieve a reversal to values significantly above vehicle-treated controls (Figure 4).

In contrast to the inactivity of thioridazine and clozapine when administered alone, sulpiride 0.1 to 5.0 mg kg^{-1} had overall a disinhibitory profile, increasing the latency of first movement from the light to the dark area and the time spent in the light area to values above vehicle controls when administered alone, or as a co-treatment with 5-HTP. Lower doses of sulpiride 0.01 and 0.05 mg kg^{-1} whilst failing to modify behaviour in their own right also antagonized (0.01 mg kg^{-1}) or reversed (0.05 mg kg^{-1}) the inhibitory effects of 5-HTP (Figure 5).

Sulpiride (0.1–10 mg kg^{-1}) was also effective in the rat in increasing social interaction when administered alone and to antagonize at 0.01 mg kg^{-1} and reverse at 0.1–10 mg kg^{-1} the inhibitory effects of 5-HTP. Thioridazine at 0.05 mg kg^{-1} had no effect in its own right of enhancing social interaction but antagonized the inhibitory effect of 5-HTP. Increasing the dose of thioridazine to 0.1, 0.5 and 1.0 mg kg^{-1} disinhibited behaviour in its own right, increasing social interaction to above vehicle control values. A disinhibitory profile was also evident with these doses of thioridazine plus 5-HTP. Clozapine

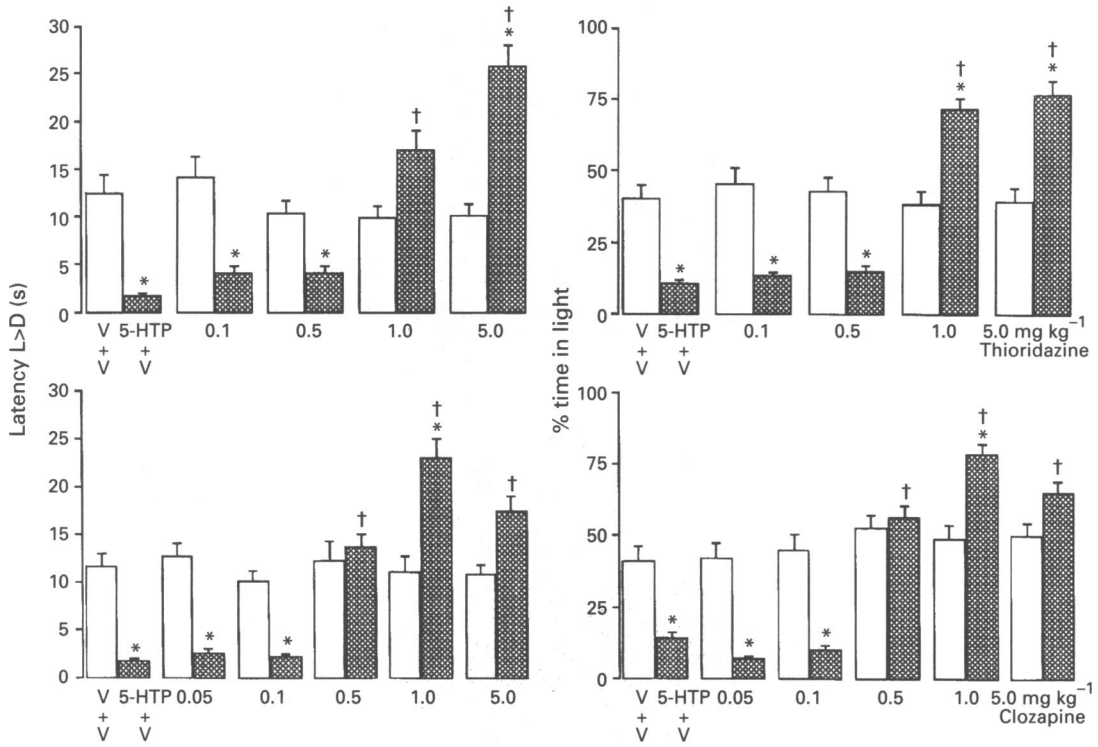

Figure 4 The effect of clozapine and thioridazine in the light/dark exploration test in the mouse. Animals received an intraperitoneal injection of vehicle (V, open columns) or 5-hydroxytryptophan (5-HTP, 50 mg kg^{-1}, cross-hatched columns) plus vehicle or clozapine or thioridazine at the doses indicated with testing 40 min after the last treatment. Latency of first movement from the light (L) to the dark (D) compartment and % time spent in the light compartment during the 5 min test period are shown. $n=10$, mean values ± s.e. mean are shown. *$P<0.05$ compared to control V+V and †$P<0.05$ compared to 5-HTP+V; (ANOVA and Dunnett's t test).

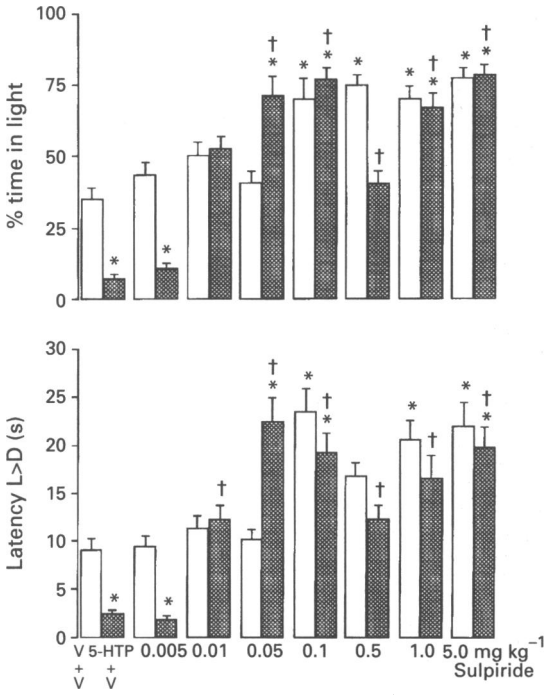

Figure 5 The effect of sulpiride in the light/dark exploration test in the mouse. Animals received an intraperitoneal injection of vehicle (V, open columns) or 5-hydroxytryptophan (5-HTP, 50 mg kg^{-1}, cross-hatched columns) plus vehicle or sulpiride (0.005–5 mg kg^{-1}) with testing 40 min after the last treatment. Latency of first movement from the light (L) to the dark (D) compartment and % time spent in the light compartment during the 5 min test period are shown. $n=10$, mean values ± s.e. mean are shown. *$P<0.05$ compared to control V+V and †$P<0.05$ compared to 5-HTP+V; (ANOVA and Dunnett's t test).

when administered alone at 0.01 to 1.0 mg kg^{-1} did not modify rat social interaction. But clozapine at 0.05 mg kg^{-1} antagonized and at 0.1 and 0.5 mg kg^{-1} reversed the inhibitory effect of 5-HTP to one of increased social interaction. The use of a higher dose of 1.0 mg kg^{-1} clozapine also antagonized the effect of 5-HTP but the trend to increase social interaction to above vehicle-treated control values failed to achieve significance (Figure 6).

The effect of the neuroleptic agents, haloperidol, benperidol and spiperone

Preliminary experiments established the doses of neuroleptic agents causing locomotor depression (i.e. a reduction in line crossings) as a consequence of dopamine receptor blockade. Subsequently, maximal doses of neuroleptic agents were selected to cover the potential range of their pharmacological activities without unduly compromising the rationale of the behaviour test procedures. Within an experiment, the effect of session for vehicle, 5-HTP, haloperidol, benperidol or spiperone on the interaction between 5-HTP and the neuroleptic agents was never significant, with ANOVA F ratios giving values where $P>0.05$.

The administration of haloperidol, benperidol and spiperone (0.04 to 0.16 mg kg^{-1}) alone failed to modify mouse behaviour in the light/dark test. They also failed to antagonize the inhibitory effects of 5-HTP. A higher dose of spiperone, haloperidol and benperidol (0.32 mg kg^{-1}) appeared to antagonize the inhibitory effects of 5-HTP by increasing the latency of onset of the first movement from the light to the dark compartment and increasing the time spent in the light compartment. However, such changes were accompanied by a marked reduction in line crossings in both the light and dark components. The reduction in line crossings indicates a non-specific reduction in mouse locomotor activity to interfere with the measurement of changes in behavioural responding to aversive situations (Figures 7 and 8).

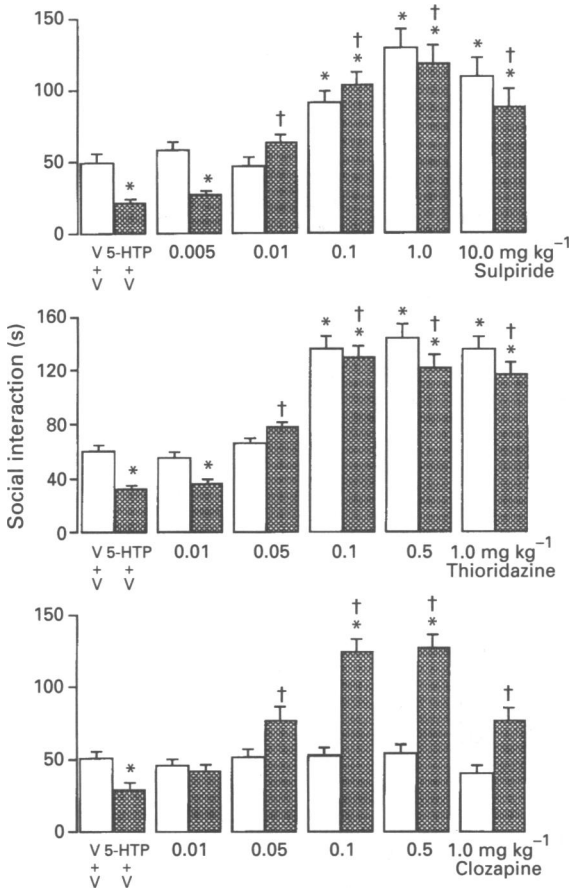

Figure 6 The effect of thioridazine, clozapine and sulpiride on social interaction in rats. Pairs of rats received an intraperitoneal injection of vehicle (V, open columns) or 5-hydroxytryptophan (5-HTP, 50 mg kg^{-1}, cross-hatched columns) plus vehicle or thioridazine, clozapine and sulpiride at the doses indicated with testing for 10 min, 40 min after the last treatment. $n=6$ pairs per group, mean values ± s.e. mean are shown. *$P<0.05$ compared to control V+V and †$P<0.05$ compared to 5-HTP+V; (ANOVA and Dunnett's t test).

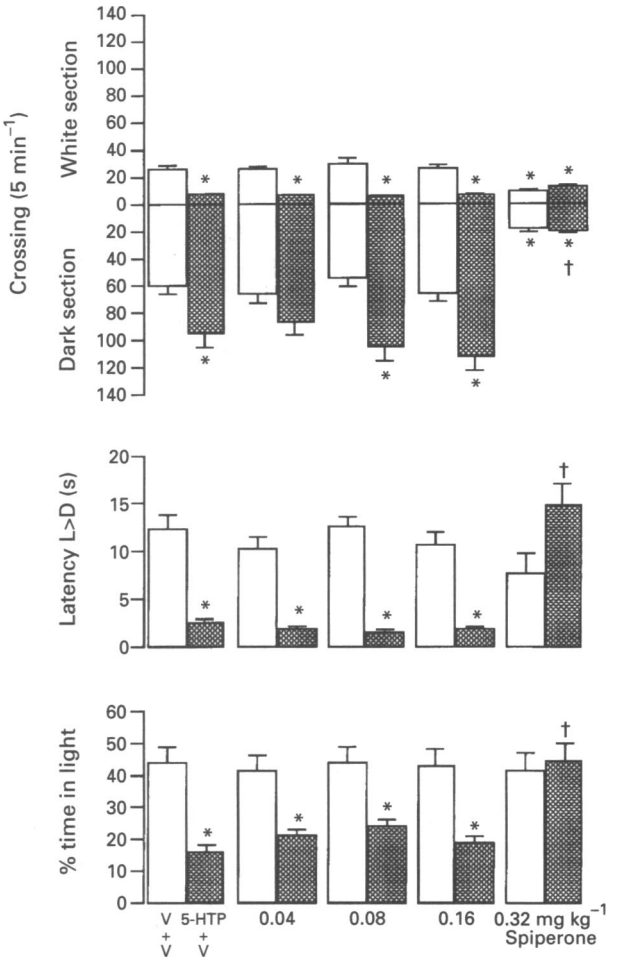

Figure 7 The effect of spiperone in the light/dark exploration test in the mouse. Animals received an intraperitoneal injection of vehicle (V, open columns) or 5-hydroxytryptophan (5-HTP, 50 mg kg^{-1}, cross-hatched columns) plus vehicle or spiperone (0.04–0.32 mg kg^{-1}) with testing 40 min after the last treatment. Latency of first movement from the light (L) to the dark (D) compartment, % time spent in the light compartment and line crossings during the 5 min test period are shown. $n=10$, mean values ± s.e. mean are shown. *$P<0.05$ compared to control V+V and †$P<0.05$ compared 5-HTP+V; (ANOVA and Dunnett's t test).

The administration of spiperone, haloperidol and benperidol (0.04 and 0.08 mg kg^{-1}) alone failed to modify social interaction in the rat. However, both doses of spiperone antagonized the inhibitory effects of 5-HTP, the values of social interaction returning to vehicle-treated controls. There was also a trend for haloperidol and benperidol at 0.04 mg kg^{-1} to attenuate the effects of 5-HTP but this did not achieve significance. This was not observed with a dose of 0.08 mg kg^{-1}. The use of the three neuroleptic agents at doses of 0.04 and 0.08 mg kg^{-1} was not associated with any significant reductions in line crossings. This contrasted with the marked reductions in line crossings caused by the administration of 0.16 and 0.32 mg kg^{-1} of spiperone, haloperidol and benperidol. The reduction in the values of social interaction to below those actually recorded for 5-HTP may reflect the marked reduction in locomotor activity caused by the higher doses of spiperone, haloperidol and benperidol (Figure 9).

Discussion

The first findings of the study established that chemically dissimilar 5-HT receptor antagonists, ketanserin, methysergide, RP62203 and MDL11939, with high affinity for the 5-HT$_{2A/2C}$ receptors, mimicked the actions of ritanserin in antagonising the inhibitory effect of 5-hydroxytryptophan in the mouse light/dark test and rat social interaction (Costall *et al.*, 1993a, b). The effectiveness of some 5-HT$_{2A/2C}$ receptor antagonists, e.g. mianserin and pizotifen, in enhancing rat social interaction in their own right, and the failure of ketanserin, a ligand with an affinity one hundred times less for the 5-HT$_{2C}$ than the 5-HT$_{2A}$ receptor, has been interpreted as reflecting the greater importance of drug action at the 5-HT$_{2C}$ receptor (Kennett, 1992). However, this distinction was not apparent in the present study since all five 5-HT$_{2A/2C}$ receptor antagonists when administered alone across an extensive dose-range failed to modify the behaviour of mice in the light/dark test or rat social interaction. This indicates an absence of an endogenous behaviourally inhibitive 5-HT tone on the 5-HT$_{2A/2C}$ receptors in the mouse and rat as assessed in the present experimental paradigms. This failure is in agreement with many literature findings (File, 1981; Niesink & Van Ree, 1982; Gardner, 1986; Deacon & Gardner, 1986) and is in concordance with the hypothesis of a silent role for the 5-HT$_2$ receptor in physiological conditions (see Leysen, 1992). But this does not negate the potential importance of the 5-HT$_2$ site in mediating inhibitory effects since *m*-chlorophenylpiperazine, other agonists at the 5-HT$_{2C}$ site and withdrawal from ethanol treatment may mediate their anxiogenic-like behaviours in animals (mouse light/dark test, rat social interaction) and anxiogenic effects in man at this site (see Kennett *et al.*, 1989; Lal *et al.*, 1993).

Evidence to support a greater involvement of the 5-HT$_{2C}$ than the 5-HT$_{2A}$ receptor comes from the present findings in

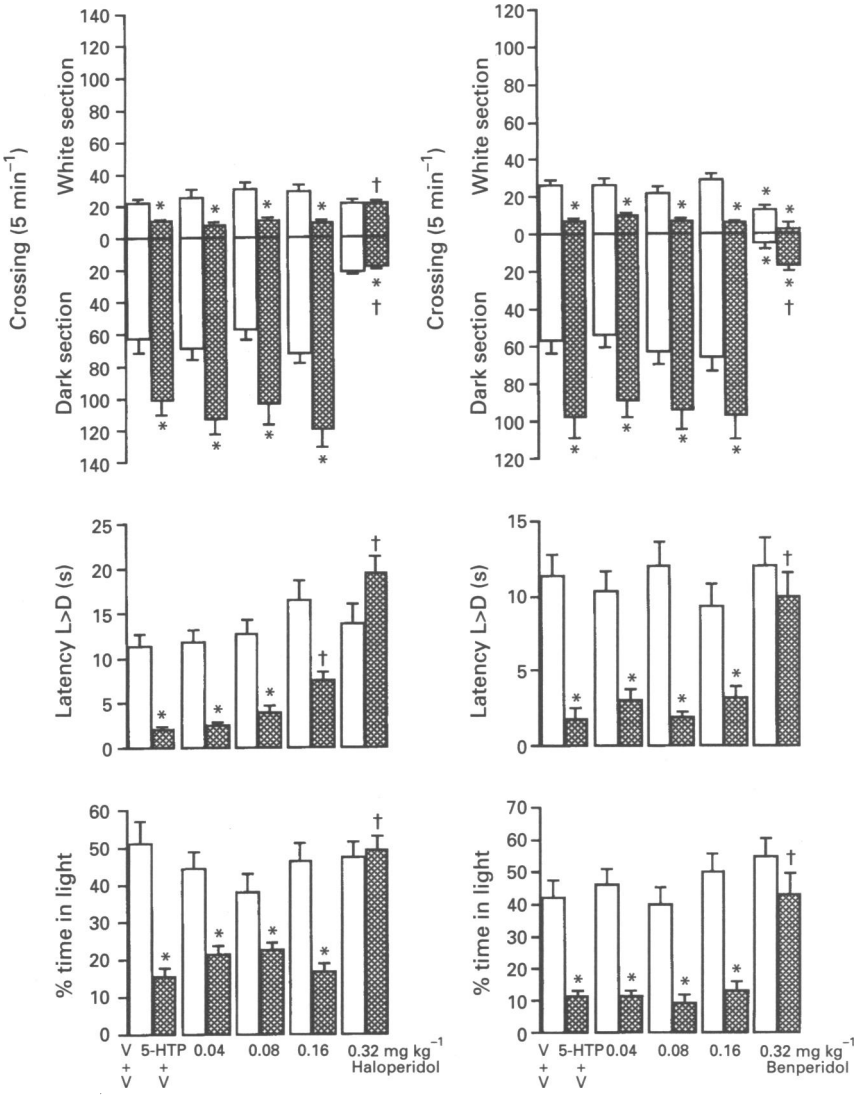

Figure 8 The effect of haloperidol and benperidol in the light/dark exploration test in the mouse. Animals received an intraperitoneal injection of vehicle (V, open columns) or 5-hydroxytryptophan (5-HTP, 50 mg kg^{-1}, cross-hatched columns) plus vehicle or haloperidol or benperidol (0.04–0.32 mg kg^{-1}) with testing 40 min after the last treatment. Latency of first movement from the light (L) to the dark (D) compartment, % time spent in the light compartment and line crossings, during the 5 min test period are shown. $n=10$, mean values ± s.e. mean are shown. *$P<0.05$ compared to control V+V and †$P<0.05$ compared to 5-HTP+V; (ANOVA and Dunnett's test).

both rat and mouse that ketanserin was approximately 100 times less potent than ritanserin in antagonizing the inhibitory effects of 5-HTP. But such support may be questioned by the data obtained with RP62203, a compound similar to ritanserin as a very high affinity ligand for the 5-HT$_{2A}$ receptor but with a 300 fold reduced affinity for the 5-HT$_{2C}$ receptor (see Doble *et al.*, 1992). Thus RP62203 retained a potency comparable to ritanserin in the mouse and was actually 100 times more potent than ritanserin in the rat. The potency of ritanserin and RP62203 in antagonizing the effects of 5-HTP in the mouse light/dark test is comparable to their potency in inhibiting the head twitches in mice induced by 5-HTP and the 5-HT$_{2A/2C}$ agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (Doble *et al.*, 1992). The marked differences in potency of RP62203 between the mouse and rat remains to be explained and may not necessarily be interpreted solely in terms of drug action at the 5-HT$_{2A/2C}$ receptors. Thus RP62203 has very high affinity for the α_1-adrenoceptor, high affinity for the 5-HT$_{1A}$ and histamine receptors and low affinity for the 5-HT$_3$ receptor (Doble *et al.*, 1992). Such interactions, particularly at the 5-HT$_{1A}$ and 5-HT$_3$ receptors which can mediate changes in behaviour in response to aversive situations, are worthy of further investigation (see Costall *et al.*, 1990; Treit, 1991).

Further evidence to question the greater involvement of the 5-HT$_{2C}$ as compared to the 5-HT$_{2A}$ receptor comes from the present use of MDL11939, being 10 and 500 times more potent even than RP62203 in the rat and mouse, with an affinity 160 fold greater for the 5-HT$_{2A}$ than the 5-HT$_{2C}$ receptor (Pierce *et al.*, 1992). MDL11939 was the most potent compound tested and is a highly specific 5-HT receptor antagonist (Dudley *et al.*, 1988). Its interaction with the 5-HT$_{2A}$ receptor may be relevant to its exceptional potency in antagonizing the inhibitory effects of 5-HTP but questions remain. Thus MDL11939 and ketanserin have virtually the same high affinity for 5-HT$_{2A}$ receptors yet their behavioural potency is at variance by a factor of 100,000. Even accepting the difficulties of translating *in vitro*-based receptor affinity data to an effect *in vivo*, the discrepancy may indicate an additional and unspecified action of MDL11939 in modifying behavioural responding to aversive situations.

Within the above perspective, it was not surprising that the atypical neuroleptic agents, clozapine and thioridazine, antagonized the inhibitory effects of 5-HTP in the mouse and rat. Indeed, the compounds were only ten times less potent than ritanserin, correlating with their high affinity for the 5-HT$_{2A}$ receptor, the affinity being only an order of magnitude less potent than ritanserin (Leysen, 1992). However, an additional action on the 5-HT$_{2C}$ receptor cannot be discounted since the

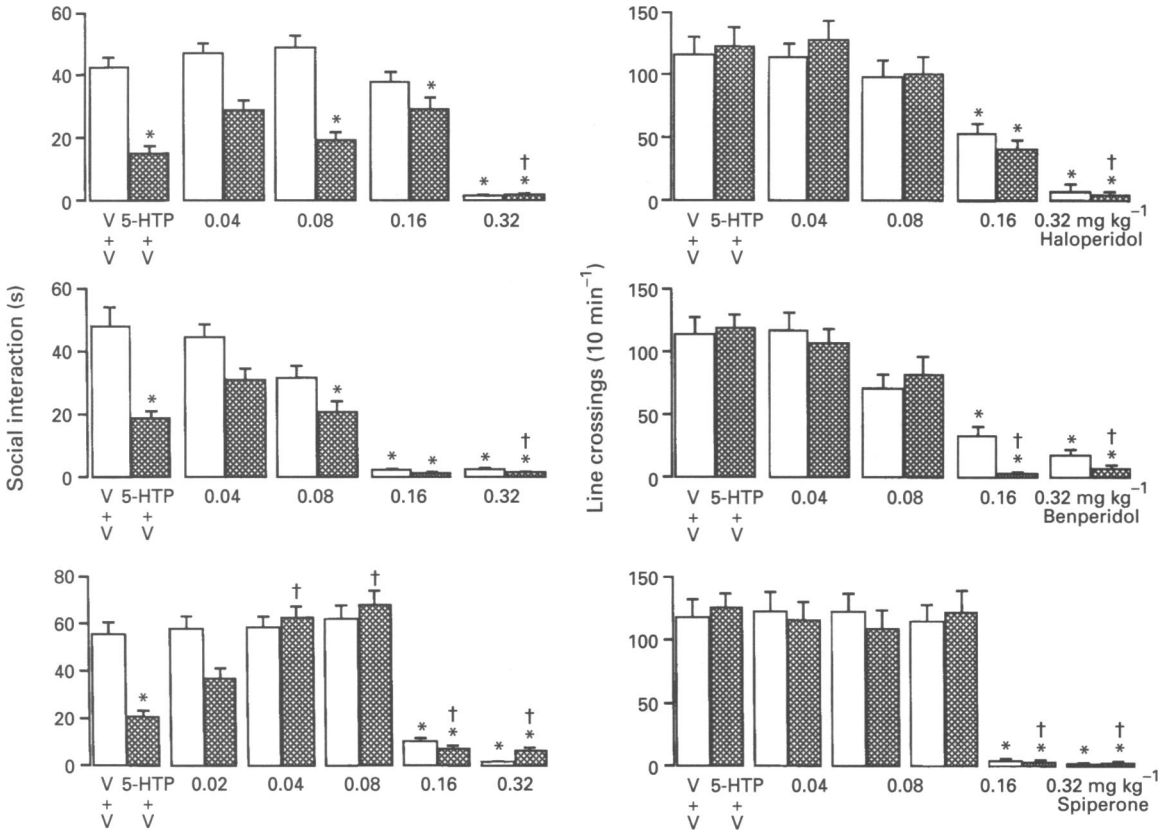

Figure 9 The effect of haloperidol, benperidol and spiperone on social interaction in rats. Pairs of rats received an intraperitoneal injection of vehicle (V, open columns) or 5-hydroxytryptophan (5-HTP, 50 mg kg^{-1}, cross-hatched columns) plus vehicle or haloperidol, benperidol or spiperone at the doses indicated with testing for social interaction and line crossings for 10 min, 40 min after the last treatment. $n=6$ pairs per group, mean values ± s.e. mean are shown. *$P<0.05$ compared to V+V; †$P<0.05$ compared to 5-HTP+V; (ANOVA and Dunnett's test).

affinity of clozapine and thioridazine is only three and twelve fold less respectively at the 5-HT_{2C} as compared to the 5-HT_{2A} receptor (Leysen, 1992). Further, clozapine has an affinity for the 5-HT_3 receptor only slightly less than for the 5-HT_{2A} receptor (see Zifa & Fillion, 1992), the affinity of clozapine and thioridazine for the 5-HT_{1A} receptor being considerably less (60 to 100 fold) (Leysen, 1992). Therefore it is likely that the affinity of clozapine and thioridazine for the 5-$HT_{2A/2C}$ receptor(s) played a major role in reversing the inhibitory effect of 5-HTP to one of disinhibition.

A disinhibitory profile was also observed in both rats and mice treated with sulpiride and 5-HTP, but this profile was also induced by the administration of sulpiride alone. This confirms previous reports using the mouse light/dark test (Costall *et al.*, 1987) and extends the findings to an effect in the rat social interaction test. Therefore, from the present studies, it remains uncertain as to whether sulpiride actually antagonizes the effect of 5-HTP or whether its inherent disinhibitory action masks the effect of 5-HTP. Since sulpiride has low affinity for the 5-HT_2 recognition site (Leysen *et al.*, 1993), an alternative mechanism is sought. It is unlikely that this relates to the affinity of sulpiride for the dopamine receptor since the disinhibitory effects of sulpiride were achieved at doses 100 fold lower than those required to antagonize dopamine-mediated behaviours (Costall & Naylor, 1976).

The ability of clozapine, sulpiride and thioridazine to antagonize the dopamine D_2 receptor is considered the major mechanism of action in antagonizing the behavioural effects of dopamine agonists in animals and to attenuate psychosis in man. The 'atypical' nature of their effects relates to a greatly reduced incidence of EPS as compared to agents such as haloperidol and, at least for clozapine and thioridazine, this may relate to a concomitant 5-HT_2 and muscarinic cholinoceptor blockade (see Costall *et al.*, 1975). It remains clear that the atypical neuroleptic agents have a consistent profile to antagonize directly or indirectly the behaviourally inhibitive effects of 5-HTP in the rodent models.

The results obtained with the atypical neuroleptic agents prompted further experiments to determine if typical neuroleptic agents may also attenuate the effects of 5-HTP. Haloperidol, benperidol and spiperone are potent dopamine receptor antagonists, with an affinity for the dopamine receptor slightly (benperidol, spiperone, 2 fold) or considerably (haloperidol, 100 fold) greater than for the 5-HT_{2A} site (Leysen, 1992; Ziffa & Fillion, 1992). Low doses of 40 and 80 μg kg^{-1} of haloperidol, benperidol and spiperone, doses known to attenuate the behavioural effects of dopamine agonists (Janssen *et al.*, 1966; Costall & Naylor, 1980), failed in the mouse to modify behaviour when administered alone or with 5-HTP. However, in the rat the low doses of spiperone (but not haloperidol or benperidol) antagonized but did not reverse the inhibitory effects of 5-HTP on social interaction. It remains possible that a reversal may have occurred with higher doses of spiperone, but the use of such doses of all three agents in both rats and mice caused locomotor depression, probably as a consequence of dopamine receptor blockade, obscuring any direct changes in responding to the aversive situations. The species differences in response to spiperone, and the complicating effects of dopamine receptor blockade, precludes any firm conclusions from the animal models as to whether or not typical neuroleptic agents directly affect behavioural responding to aversive situations. But such information is available from human studies.

In man, anxiety is a symptom common to many syndromes and in schizophrenia, neuroleptic therapy reduces anxiety concomitant with alleviation of the psychosis. It has been generally considered that the reduction in anxiety is a consequence of the reduction in psychosis, but it should be noted

that the disinhibitory effects of clozapine in man can be observed in some patients with no improvement in positive symptoms (Meltzer, 1991). That neuroleptic agents may directly influence anxiety or disinhibit behaviour is known from their substantial use in the treatment of non-psychotic illness. Thus typical and atypical neuroleptic agents are widely used in the treatment of affective disorders, anxiety, restlessness and agitation. For example, sulpiride reduces anxiety in neuroses (Kawano *et al.*, 1975; Muzio *et al.*, 1975; Takagi *et al.*, 1975; Standish-Barry *et al.*, 1983), the use of flupenthixol in neurotic depression is as effective as diazepam to alleviate anxiety (Johnson, 1983) and thioridazine was one of the first neuroleptics to be successfully used in the treatment of depression (see Robertson & Trimble, 1982).

The major finding of the present study has been to demonstrate a functional disinhibitory potential or effect of atypical neuroleptic agents in animal models that may serve for the better understanding of the nature of the disinhibitory effects of neuroleptic agents in man. A 5-HT_2 receptor antagonism of thioridazine, clozapine and other neuroleptic agents may contribute to such effects and there is an extensive literature attesting to a role for 5-HT in anxiety, depression and motor behaviour (see Vanderwolf, 1989; Zuardi, 1990; Lund & Mjellum, 1993) and a more restricted literature in schizophrenia (see review by Costall & Naylor, 1991). But one of the most interesting findings of the present study is the absence of a correlation between the behavioural effects of the drugs and their affinity for the 5-HT_{2A} and 5-HT_{2C} receptors, from which the possibility of an involvement with other receptor mechanisms may be inferred. For example, the 5-HT_2 receptor has recently been shown to accommodate a third subtype, the 5-HT_{2B} receptor; the mRNA transcript for this receptor is present in the mouse and possibly the rat brain (see review by Baxter *et al.*, 1995). *m*-Chlorophenyl piperazine has partial agonist action at the 5-HT_{2B} receptor (Baxter *et al.*, 1995) and the first selective 5-$HT_{2C/2B}$ receptor antagonist SB200646A facilitates rat social interaction and antagonizes the anxiogenic-like activity of *m*-chlorophenylpiperazine (Kennett *et al.*, 1994a, b). Ritanserin has similar high affinity for the 5-$HT_{2A,2B}$ and $_{2C}$ receptors and more selective 5-HT_{2B} receptor ligands will be required to establish the role of this receptor in behavioural responding to aversive situations. It remains an interesting observation that in the present experiments, spiperone was effective in the rat but not in the mouse in antagonizing the inhibitory effects of 5-hydroxytryptophan, whereas the affinity of spiperone for the 5-HT_{2B} receptor is approximately 50 fold greater in the mouse than in the rat (Baxter *et al.*, 1995).

Further possibilities relate to an action on a novel 5-HT receptor designated 5-HT_7 which has been identified as a candidate to mediate 5-HT-induced phase shifts in neuronal activity (Lovenberg *et al.*, 1993). Ritanserin and methysergide have nanomolar affinity for this receptor and it would be of value to assess the effect of neuroleptic agents in this system. Attempts to establish the mechanisms mediating the disinhibitory effects of thioridazine and sulpiride when administered alone are also of importance.

Finally, the variability in results of studies on the administration of the 5-HT_2 receptor antagonists alone in rodent models of anxiety may reflect differences in the degree of 5-HT tone on inhibitory and disinhibitory mechanisms. Such differences may offer further clues as to the physiological role of 5-HT in behavioural responding, its possible role in the pathology of inhibited behaviour and the design of improved treatments for schizophrenia.

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