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The influence of 5-HT_2 and 5-HT_4 receptor antagonists to modify drug induced disinhibitory effects in the mouse light/dark test

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1 The ability of 5-HT_2 and 5-HT_4 receptor antagonists to modify the disinhibitory profile of diazepam and other agents was investigated in male BKW mice in the light/dark test box.

2 The 5-$HT_{2A/2B/2C}$ receptor antagonists ritanserin, MDL11939 and RP62203 and also methysergide, which failed to modify mouse behaviour when administered alone, caused dose-related enhancements (4 to 8 fold) in the potency of diazepam to disinhibit behavioural responding to the aversive situation of the test box.

3 Ritanserin was shown to enhance the disinhibitory potency of other benzodiazepines, chlordiazepoxide (4 fold), temazepam (10 fold) and lorazepam (10 fold), the 5-HT_{1A} receptor ligands, 8-OH-DPAT (25 fold), buspirone (100 fold) and lesopitron (500 fold), the 5-HT_3 receptor antagonists, ondansetron (100 fold) **R**(+)-zacopride (100 fold) and **S**(−)-zacopride (greater than a 1000 fold), the substituted benzamides, sulpiride (10 fold) and tiapride (5 to 10 fold) and the cholecystokinin $(CCK)_A$ receptor antagonist, devazepide (100 fold). It also reduced the onset of action of disinhibition following treatment with the 5-HT synthesis inhibitor parachlorophenylalanine. Ritanserin failed to enhance the disinhibitory effects of the CCK_B receptor antagonist CI-988, the angiotensin AT_1 receptor antagonist losarten or the angiotensin converting enzyme inhibitor ceranapril.

4 The 5-HT_4 receptor antagonists SDZ205-557, GR113808 and SB204070 caused dose-related reductions in the disinhibitory effect of diazepam, returning values to those shown in vehicle treated controls. The antagonists failed to modify mouse behaviour when administered alone.

5 GR113808 was also shown to cause a dose-related antagonism of the disinhibitory effects of chlordiazepoxide, lorazepam, 8-OH-DPAT, buspirone, lesopitron, ondansetron, **R**(+)-zacopride, sulpiride, tiapride, devazepide, CI-988, losarten, ceranapril and parachlorophenylalanine.

6 It was concluded that in BKW mice (a) the failure of 5-HT_2 and 5-HT_4 receptor antagonists when administered alone to modify behaviour in the light/dark test indicates an absence of an endogenous 5-HT tone at the 5-HT_2 and 5-HT_4 receptors and (b) the enhancement by the 5-HT_2 receptor antagonists and attenuation by the 5-HT_4 receptor antagonists of drug-induced disinhibition indicates a plurality of 5-HT receptor involvement in the mediation of drug-induced disinhibitory profiles in the mouse.

Keywords: 5-HT_2 and 5-HT_4 receptors; benzodiazepines; anxiolytic-like agents; disinhibition

Introduction

Brodie and Shore (1957) were the first to hypothesize that behavioural arousal is a result of catecholaminergic excitation and 5-hydroxytryptaminergic inhibition. Subsequently, supportive evidence of a behavioural inhibitory role for 5-hydroxytryptamine (5-HT) came from the use of selective neurotoxic lesioning of the 5-hydroxytryptaminergic systems and from the inhibition of 5-HT synthesis to enhance punished responding and cause behavioural disinhibition in animal models of anxiety (see reviews by Iversen, 1984; Gardner, 1986; Chopin & Briley, 1986; Nutt & George, 1989). The hypothesis gained further support from pharmacological findings that (a) 5-HT_2 or 5-HT_3 receptor blockade by ritanserin, ondansetron and other agents has a disinhibitory profile in animal models of anxiety (Critchley & Handley, 1987; Costall & Naylor, 1991; Kennett, 1992), (b) 5-hydroxytryptophan and the 5-$HT_{2A/2C}$ receptor agonist m-chlorophenylpiperazine have anxiogenic profiles of action in animal models and man (see Kennett *et al*., 1989), (c) the 5-HT_{1A} receptor agonist effects of buspirone and other agents at somatodendritic receptors located on 5-HT cells reduce cell firing, 5-HT release, disinhibit behaviour in animals and are anxiolytic in man (see review in Treit, 1991; Sprouse & Aghajanian, 1986; Barnes *et al*., 1992b) and (d) benzodiazepines are reference anxiolytic agents and have been shown to reduce 5-HT release (Wise *et al*., 1972; Barnes *et al*., 1992b). However, support for the hypothesis of a singular role for 5-HT to inhibit behaviour is qualified in a number of ways.

Firstly, there are many inconsistencies in the literature; numerous studies have shown that 5-HT_{1A}, 5-$HT_{2A/2B/2C}$ and 5-HT_3 receptor ligands fail to inhibit or disinhibit behaviour in animal models of anxiety (see Kennett, 1992 and reviews by Thiebot, 1986; Gardner, 1986; Treit, 1991; Costall & Naylor, 1991; Barrett & Vanover, 1993). That such treatments exert a highly effective blockade of 5-HT receptors or 5-HT function in the presence of a variable release of response suppression, contrasts with the consistent disinhibitory behavioural effects of the benzodiazepines achieved even after destruction of the 5-hydroxytryptaminergic neurones or 5-HT receptor blockade (Cook & Sepinwall, 1975; Thiebot *et al*., 1984). Such findings are at variance with a role for 5-HT to inhibit behaviour.

But secondly, there is preliminary evidence of a complex interaction between the effect of drug treatments and the 5-HT system that may indicate both an inhibitory and disinhibitory role for 5-HT. Thus, the precursor of 5-HT, 5-hydroxytryptophan, has been shown in both the Montgomery and Vogel conflict tests to produce a biphasic dose-response curve, an anxiolytic-like action at low doses and an anxiogenic-like action at high doses (Söderpalm & Engel, 1990). It was suggested that the latter response may derive from increased 5-HT neurotransmission through unspecified 5-HT receptors; the anxiolytic-like action remained unexplained. Relevant to such observations, the use of ritanserin has (a) tentatively identified 5-$HT_{2A/2B/2C}$ receptors as mediating the inhibitory actions of 5-hydroxytryptophan in the mouse light/dark test and rat social interaction and (b) revealed a consistent disinhibitory action of 5-hydroxytryptophan that was normally masked by the inhi-

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bitory effect (Costall *et al*., 1993a; Cheng *et al*., 1994). Furthermore, ritanserin has been shown to enhance the potency of diazepam and reveal the effect of **S**(−)-zacopride to disinhibit behaviour in the mouse light/dark test (Costall & Naylor, 1994a,b).

From these experiments a tentative identification of the 5-HT receptor mediating the disinhibitory effects of the ritanserin/5-hydroxytryptophan interaction and diazepam was made by use of the mixed 5-HT_3/5-HT_4 receptor antagonists tropisetron and SDZ205-557 (Eglen *et al*., 1994). The latter agents, but not the selective 5-HT_3 receptor antagonist ondansetron, were found to antagonize the disinhibitory effects of the ritanserin/5-hydroxytryptophan interaction and diazepam treatments (Costall *et al*., 1993b; Cheng *et al*., 1994): the 5-HT_4 receptor was hypothesized to mediate the disinhibitory effects. The data indicate that a raised 5-HT function may have the potential to mediate either a disinhibitory or inhibitory profile which is at variance with the original hypothesis of a singular role for 5-HT to inhibit behaviour.

In the present study, in order to investigate further a 5-HT_2/5-HT_4 receptor involvement in drug-induced changes in behavioural responding to an aversive situation, we have used a range of 5-HT_2 and 5-HT_4 receptor antagonists to determine whether the ability of ritanserin to enhance and tropisetron and SDZ205-557 to reduce the disinhibitory potency of diazepam can (a) also be detected when other 5-HT_2/5-HT_4 receptor antagonists are used and (b) be extended to the actions of other pharmacological agents with disinhibitory profiles in the mouse light/dark test.

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 ($21 \pm 1^{\circ}C$) and controlled lighting (dark period 07 h00 min–19 h00 min). The cleaning and feeding of animals was performed at fixed periods by specified animal husbandry staff. Mice were allowed free access to water and fed *ad libitum* on a CRM high protein diet obtained from Special Diet Services.

Behavioural testing of mice

Mice were removed from the dark holding rooms and placed into a dark container for transportation at 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 08 h00 min and 13 h00 min 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 m 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 (1×60W, 0 Lux) and partitioned from the remainder of the box which was painted white and brightly illuminated with a 1×60W (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. Forty minutes after the final drug administration, mice were placed into 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 latency of the initial movement from the light to the dark area, (b) the time spent in the light and dark areas, (c) the number of exploratory rearings in the light and dark sections and (d) the number of line crossings in the light and dark sections.

Experimental design

Naïve mice were used in all experiments. Animals received vehicle or drug treatments intraperitoneally with 40 min between treatments and behavioural testing 40 min after the last treatment. An exception was in the experiment with parachlorophenylalanine; animals received intraperitoneally a 1, 2 or 3 day treatment with 200 mg kg^{-1} parachlorophenylalanine (given as a single daily injection) with the administration of ritanserin 24 h following withdrawal from the 1, 2 or 3 day treatment and behavioural testing after 40 min.

Preliminary experiments were used to establish the range of doses of the various drug treatments required to demonstrate an interaction with the 5-HT_2 and the 5-HT_4 receptor antagonists.

The disinhibitory agents were administered at 4 to 7 dose levels causing effects ranging from nil to maximum, with or without a 5-HT_2 receptor antagonist, together with a vehicle or 5-HT_2 receptor antagonist control. In the 5-HT_4 receptor antagonist interactions, a dose of the disinhibitory agent was selected as one causing a maximal effect and this was administered alone and in combination with the 5-HT_4 receptor antagonist plus a vehicle control.

Such designs necessitated the testing of animals on a number of days, each drug and vehicle treatment being equally represented on each day. All data were initially subject to analysis of variance with factors of treatment and session to establish the absence of an interaction within treatments and between sessions. F ratios indicated that such interactions were not significant ($P > 0.05$). Subsequently the data were collapsed across sessions and the significance of differences between treatments established by use of two-way ANOVA followed by Dunnett's t test.

Variation in response was minimized by closely controlled in-house breeding of animals, consistent procedures and personnel for animal husbandry, 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 (see Brett & Pratt, 1990; File *et al*., 1992; Andrews & File, 1993).

Drugs

Buspirone hydrochloride (Bristol Myers), ceranapril (Squibb), devazepide hydrochloride (Parke Davis), CI-988 (PD134308, 4-{(2-[[3-1H-indol-3-yl)-2-methyl-1-oxo-2-[[(tricyclo[3,3,1,1,3,7]dec-2-yloxy)carbonyl]amino]propyl]amino]1-phenylethyl]amino-4-oxo [R-(R*,R*)]-butanoate N-methyl]-D-glucamine) (Parke Davis), losarten potassium salt (E.I. Dupont de Nemours and Co.), GR113808 (([1-[2-methylsulphonylamino)ethyl]-4-piperidinyl]methyl-1-methyl-1H-indole-3-carboxylate maleate (Glaxo), 8-OH-DPAT (8-hydroxy-2-(di-n-propylamino)tetralin hydrochloride) (RBI), lesopitron hydrochloride (Esteve), ondansetron hydrochloride dihydrate (Glaxo), **R**(+)- and **S**(−)-zacopride (A.H. Robbins), lorazepam hydrochloride (Sigma), SB204070 (8-amino-7-chloro-(N-butyl-4-piperidyl)-methylbenzo-1,4-dioxan-5-carboxylate hydrochloride (Smith-Kline & Beecham), SDZ205-557 (2-methoxy-4-amino-5-chloro-benzoic acid 2-(dethylamino) ethyl ester) (Sandoz) and tiapride hydrochloride (Laboratoires Delagrange) were dissolved in distilled water. Sulpiride hydrochloride (Laboratoires Delagrange) was dissolved in a minimum quantity of HCl and prepared to volume with distilled water. RP62203 ((2-[3-(4-(4-flurophenyl)-piperazinyl) propyl] naphto [1,8-cd] isothiazole-1,1-dioxide)) (Rhône-Poulenc Rorer) and MDL11939 ((±)-α-phenyl-1-(2-phenylethyl)-4-piperidine methanol) (Marion Merrell Dow) were dissolved in a minimum amount of acetic acid, dilutions being prepared with saline. Ritanserin hydrochloride (Janssen Pharmaceutica) was dissolved in a 10% solution of polyethylene glycol and dilutions made with saline. Parachlorophenylalanine methyl ester (PCPA, Sigma) was prepared in a 2% solution of carboxymethylcellulose and diazepam (RBI) was dissolved in a minimum amount of

polyethylene glycol and prepared to volume with distilled water. All drugs and vehicle injections were administered intraperitoneally in a volume of 1 ml per 100 g body weight with doses expressed as the base.

Results

General observations

The differential size and intensity of illumination of the light and dark areas of the test box ensured that vehicle treated mice when placed into the aversive situation of the light area would move within approximately 9 to 12 s into the dark compartment, subsequently spending approximately 45% of their time in the light area within the 5 min test period. Also, mice showed a reduced level of line crossings of approximately 20 to 35 per 5 min in the light as compared to 55 to 75 per 5 min in the dark and rearing of 25 to 40 per 5 min in the light increased to 50 to 70 per 5 min in the dark. It should be noted that mice showed a consistent level of basal performance during the period of the present study. For example, the vehicle treated control values for the latency of first movement from the light to the dark area of the test box, in 40 groups of animals were in the range 8.6 ± 0.93 to 12.3 ± 0.82 s (10.3 ± 0.19 s, mean $\pm$ s.e.mean, $n = 400$). The consistency of response ensures a comparability of data between treatments and experiments. This is particularly important since the degree of drug-induced changes in behavioural responding to aversive situations is known to be dependent on basal levels (Jones *et al*., 1988; Andrews & File, 1993). It is also important to note that the level of basal respondings allows for both disinhibitory and inhibitory drug-induced effects (see Cheng *et al*., 1994).

Without exception, drug treatments causing a significant increase in the time spent in the light were associated with a significant increase in the latency of first movement from the light to the dark compartment. Also, drug treatments causing a significant increase in the time spent in one compartment were associated with selective increases in rearings and line crossings in that area. Conversely, a significant increase in the time spent in the dark compartment was always associated with a significant decrease in the latency of the first movement from the light to the dark compartment. An example of drug action to modify % time in the light area, the latency of first movement from the light to the dark compartment, and line crossings in both areas is shown in Figure 5. To enable a more concise presentation of data, results are generally given as the latency of first movement from the light to the dark compartment. Non-specific changes in motor performance are reflected by non-selective changes in line crossings and rearings in the two areas: such changes were not observed with the described drug treatments unless stated otherwise.

The interaction between diazepam and 5-HT$_2$ receptor antagonists

In four experiments the administration of higher doses of diazepam (0.64 or 1.25 mg kg^{-1}) alone enhanced at least two fold the latency of first movement from the light to the dark compartment (Figure 1). Such changes were associated with significant increases in the time spent in the light area and a selective increase in the rearings and line crossings within this section (data not shown). The values recorded after the administration of lower doses of diazepam (0.04 – 0.32 mg kg^{-1}) were not significantly different from those shown by vehicle treated controls. The administration of ritanserin (1.0 mg kg^{-1}), RP62203 (0.1 mg kg^{-1}), MDL11939 (0.1 mg kg^{-1}) and methylsergide (5 mg kg^{-1}) alone failed to modify mouse behaviour in the light/dark test (Figure 1). However, all these agents administered as a pretreatment enhanced the potency of diazepam to disinhibit behaviour, a dose of 0.08 mg kg^{-1} increasing the latency of first movement from the light to the dark area to a level normally observed to the administration of diazepam alone at 0.64 mg kg^{-1}. However, none of the 5-HT receptor antagonist pretreatments increased the effects of diazepam to levels above those normally recorded to the administration of diazepam alone.

In experiments designed to determine the potency of ritanserin, RP62203, MDL11939 and methysergide to enhance the disinhibitory potential of diazepam, a dose of diazepam (0.16 mg kg^{-1}) was selected which in its own right failed to modify mouse behaviour in the light dark box. Dosage regimens of ritanserin (0.5 mg kg^{-1}), RP62203 (0.1 mg kg^{-1}), MDL11939 (0.0001 mg kg^{-1}) and methysergide (0.5 mg kg^{-1}) that failed to modify mouse behaviour in their own right were the smallest doses used to precipitate a disinhibitory profile to treatment with diazepam. The intensity of the behavioural

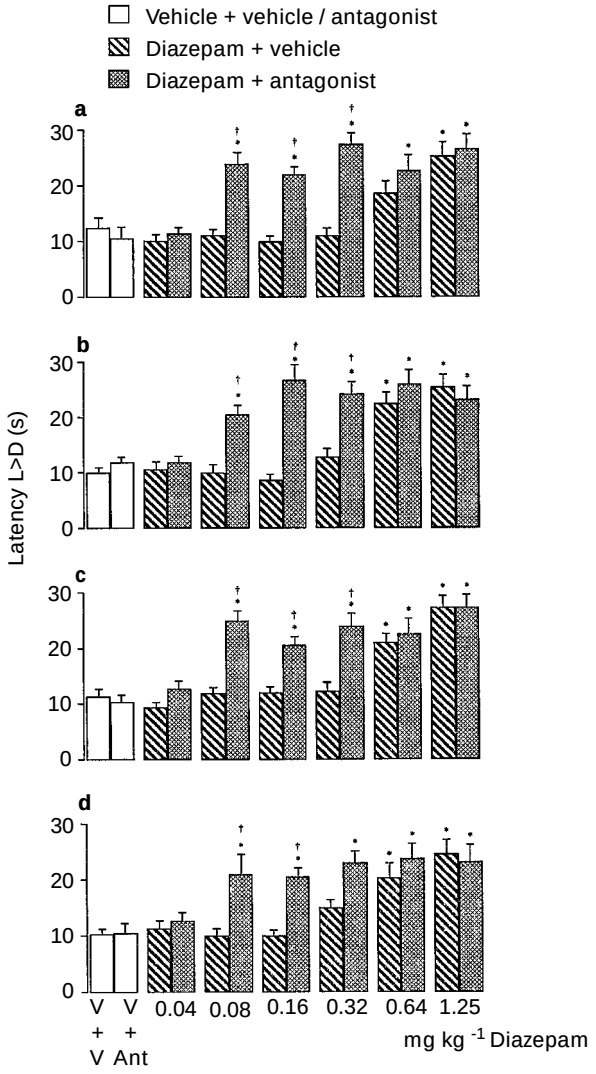

Figure 1 The ability of the 5-HT$_2$ receptor antagonists (a) ritanserin (1.0 mg kg^{-1}), (b) RP62203 (0.1 mg kg^{-1}) and (c) MDL11939 (0.1 mg kg^{-1}) and also (d) methysergide (5.0 mg kg^{-1}) to enhance the disinhibitory potency of diazepam in the mouse light/dark test. Animals received an intraperitoneal injection of vehicle (V, for diazepam) + vehicle (V, for 5-HT receptor antagonists), vehicle + 5-HT receptor antagonist (Ant) and diazepam + vehicle or 5-HT receptor antagonist. Animals received vehicle or drugs as 40 min pretreatments with testing 40 min after the last administration. Data are presented as the latency of first movement from the light to the dark compartment during the 5 min test period. Each value is the mean $\pm$ s.e.mean, $n = 10$. A significant increase in responding compared with V + V is shown as $^*P < 0.01$; a significant increase in responding in the diazepam + 5-HT receptor antagonist treated group compared to diazepam + vehicle at any one dose level is shown as $\dagger P < 0.01$ (ANOVA followed by Dunnett's t test).

change to increase the latency of first movement from the light to the dark compartment was associated with an increase in the time spent and rearings and line crossings in the light area (data not shown). The intensity of the behavioural change, equivalent to that induced by the administration of a maximally effective dose of diazepam alone was maintained but not increased with increasing dose of the 5-HT_2 receptor antagonists (Figure 2).

The interactions between diazepam and 5-HT_4 receptor antagonists

Preliminary experiments established that the selective 5-HT_4 receptor antagonists GR113808 and SB204070 were extremely potent at antagonizing the disinhibitory effects of diazepam (0.5 mg kg^{-1}). A ten thousand fold dose-range of the 5-HT_4 receptor antagonists (0.001 to 10 μg kg^{-1}) encompassed the profile of response, a reduction in the effect of diazepam persisting across a wide dose range (Figure 3). It should be noted that whilst the use of both GR113808 and SB204070 was finally extended to doses one hundred fold greater than those required to antagonize the disinhibitory effects of diazepam, the latency values still returned to those shown by the vehicle treated controls; there was no evidence that the use of the 5-HT_4 receptor antagonists could reveal an 'inhibitory' profile for diazepam. Similar comments would apply to the use of the 5-HT_4/5-HT_3 receptor antagonist SDZ205-557, which again caused a dose-related reduction of the diazepam-induced increase in latency with values returning to those of vehicle-treated control animals (Figure 3).

The interaction of ritanserin (1.0 mg kg^{-1}) with disinhibitory agents in the mouse light/dark test

The ability of ritanserin to enhance the actions of diazepam was extended in further studies to assess the breadth of action of its facilitatory effects. Other benzodiazepines were tested to

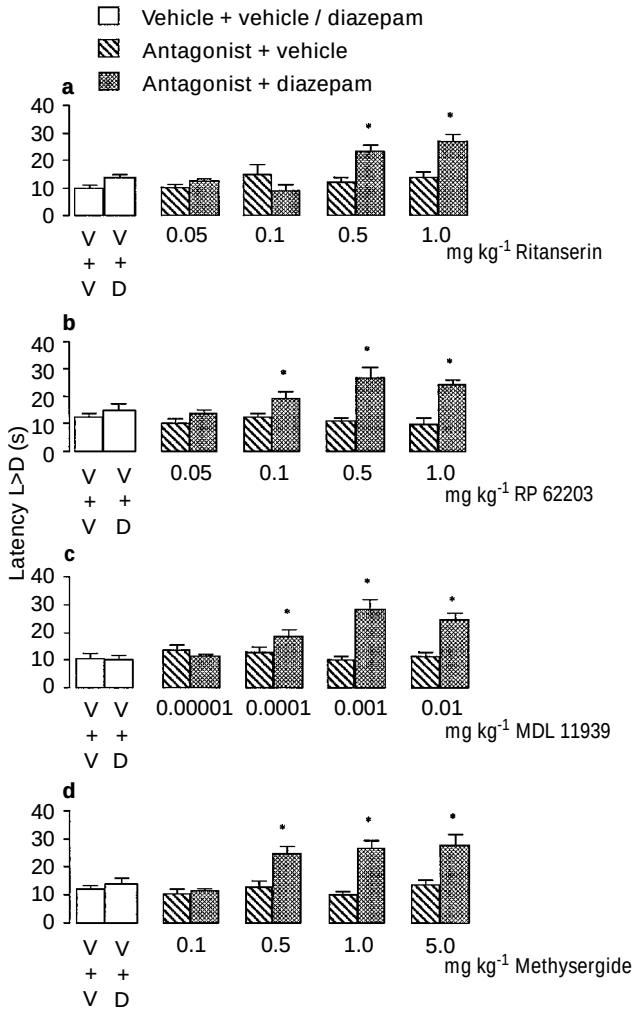

Figure 2 The dose-related actions of the 5-HT_2 receptor antagonists (a) ritanserin, (b) RP62203 and (c) MDL11939 and also (d) methysergide to reveal the disinhibitory effect of diazepam (0.16 mg kg^{-1}) in the mouse light/dark test. Animals received an intraperitoneal injection of vehicle (V, for diazepam) + vehicle (V, for 5-HT receptor antagonist), vehicle + diazepam (D) or 5-HT receptor antagonist + vehicle or diazepam. Animals received vehicle or drugs as 40 min pretreatments with testing 40 min after the last administration. Data are presented as the latency of first movement from the light to the dark compartment during the 5 min test period. Each value is the mean ± s.e.mean, $n = 10$. A significant increase in responding compared with V + V is shown as $^*P < 0.01$ (ANOVA followed by Dunnett's t test).

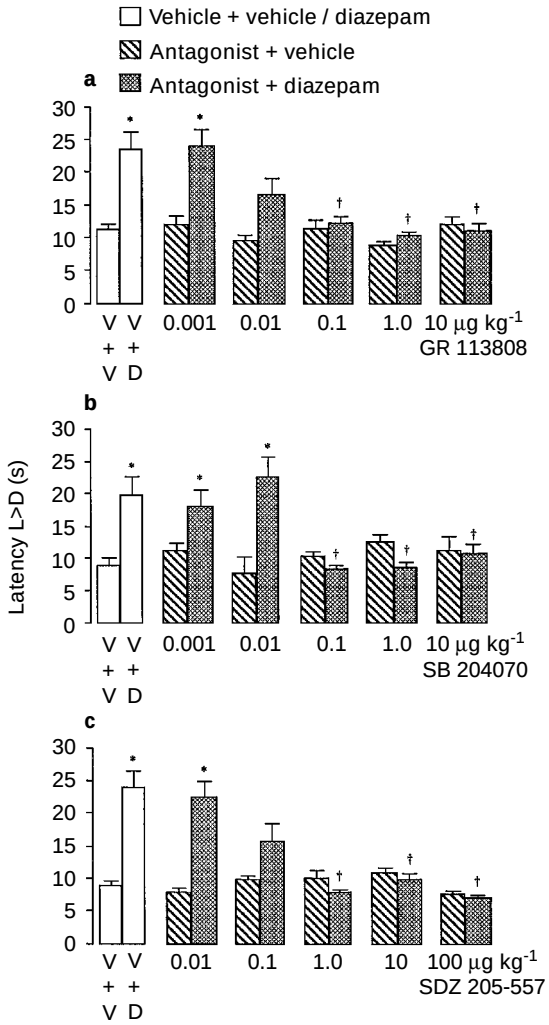

Figure 3 Antagonism by the 5-HT_4 receptor antagonists (a) GR113808, (b) SB204070 and SDZ205-557 of the disinhibitory effect of diazepam (D, 0.5 mg kg^{-1}) in the mouse light/dark test. Animals received an intraperitoneal injection of vehicle (V) + vehicle, vehicle + diazepam or 5-HT_4 receptor antagonist plus vehicle or diazepam. Animals received vehicle or drugs as 40 min pretreatments with testing 40 min after the last administration. Data are presented as the latency of first movement from the light to the dark compartment during the 5 min test period. Each value is the mean ± s.e.mean, $n = 10$. A significant increase in responding compared with V + V is shown as $^*P < 0.01$; a significant decrease in responding in the diazepam + 5-HT receptor treated group compared to vehicle + diazepam is shown as $\dagger P < 0.01$ (ANOVA followed by Dunnett's t test).

ensure that the ritanserin/diazepam interaction was not solely diazepam-dependent and other agents were selected with differing pharmacological effects but with common profiles of action to disinhibit mouse behaviour in the light dark test.

Benzodiazepines The administration of chlordiazepoxide (0.16–2.5 mg kg^{-1}) and temazepam (0.001–1.0 mg kg^{-1}) caused dose-related and maximal disinhibitory effects; they increased the latency of first movement from the light to the dark section and the time spent in the light area (Figure 4). In the presence of ritanserin mice were more sensitive to the disinhibitory effects of chlordiazepoxide and temazepam by orders of 4 and 10 fold, respectively. In the presence of ritanserin, mice also appeared 10 times more sensitive to the actions of lorazepam, although comparisons were made difficult by the modest changes caused by lorazepam when administered alone and the development of sedation at 0.5 mg kg^{-1} (line crossings in the light and dark areas in vehicle treated mice were 23 ± 3.4 per 5 min period and 56 ± 6.2 per 5 min period, respectively, and 8.6 ± 1.1 per 5 min period and 3.5 ± 1.2 per 5 min period in lorazepam treated mice) which obscured specific changes in behaviour to the aversive situation.

5-HT_{1A} receptor ligands The administration of 8-OH-DPAT (0.125 mg kg^{-1}), lesopitron (0.005 mg kg^{-1}) and buspirone (1.0 mg kg^{-1}) alone significantly disinhibited mouse behaviour in the light/dark test box, increasing the latency of first movement from and time spent in the light area. Co-treatment with ritanserin caused differential enhancements in the response to the three agents, the mice being 25, 200 and 500 times more sensitive to the disinhibitory effects of 8-OH-DPAT, buspirone and lesopitron, respectively (Figures 5 and 6). These changes in sensitivity to the disinhibitory effects of the 5-HT_{1A} receptor ligands were not accompanied by any other overt changes in behaviour. Higher doses of 8-OH-DPAT (1.0 mg kg^{-1}) and buspirone (2.0 mg kg^{-1}) caused non-selective reductions in line crossings in both the light and dark areas, which correlated with the behavioural observation of sedation.

5-HT_3 receptor ligands The administration of ondansetron (0.001 mg kg^{-1}) and **R**(+)-zacopride (0.0001 mg kg^{-1}) alone disinhibited mouse behaviour in the test box, increased doses having a comparable effect. **S**(−)-zacopride (0.00001–1.0 mg kg^{-1}) was ineffective. After pretreatment with ritanserin, the response to ondansetron and **R**(+)-zacopride was enhanced by 2 orders of magnitude with respect to both the changes in latency of movement from the light to the dark area and the time spent in the light area. The effect of the interaction between ritanserin and **S**(−)-zacopride was actually to reveal a disinhibitory potential for **S**(−)-zacopride, it being as efficacious as **R**(+)-zacopride, although a thousand times less potent. The failure of **S**(−)-zacopride alone to modify mouse behaviour in the test box precluded measurements of increases of drug potency in the presence of ritanserin. However, this was at least a thousand fold (Figure 7).

Substituted benzamide derivatives Sulpiride, in doses of 0.01 mg kg^{-1} and greater, and tiapride, in doses of 1.0 mg kg^{-1} and greater, when administered alone significantly increased the latency of the first movement from the light to the dark area and the time spent in the light area of the test box. The response to sulpiride was enhanced by ritanserin pretreatment, indicating a 10 fold increase in sensitivity to the disinhibitory effects of sulpiride. The response to tiapride enhanced by ritanserin pretreatment achieved significance at 0.1 and 0.5 mg kg^{-1} tiapride, with respect to both the latency of first movement and time spent in the light area. The data indicate an approximate 5 to 10 fold increase in potency (Figure 8).

CCK receptor antagonists The doses of CI-988 (0.001–0.1 mg kg^{-1}) required to disinhibit mouse behaviour in the test box, whether administered alone or as a co-treatment with ritanserin, were indistinguishable, with respect to measurements taken of the latency of first movement from the light to the dark area or the time spent in the light area. Devazepide (0.1 mg kg^{-1}) also disinhibited behaviour and was as efficacious as CI-988 but was 100 fold less potent when administered alone. In the presence of ritanserin the sensitivity to devazepide was increased 10 to 100 fold and at 0.001 mg kg^{-1} devazepide there was a significant difference between the two treatments (Figure 8).

Losarten and ceranapril The potency of the angiotensin receptor antagonist losarten (0.0001–0.1 mg kg^{-1}) to disinhibit mouse behaviour when administered alone or with ritanserin

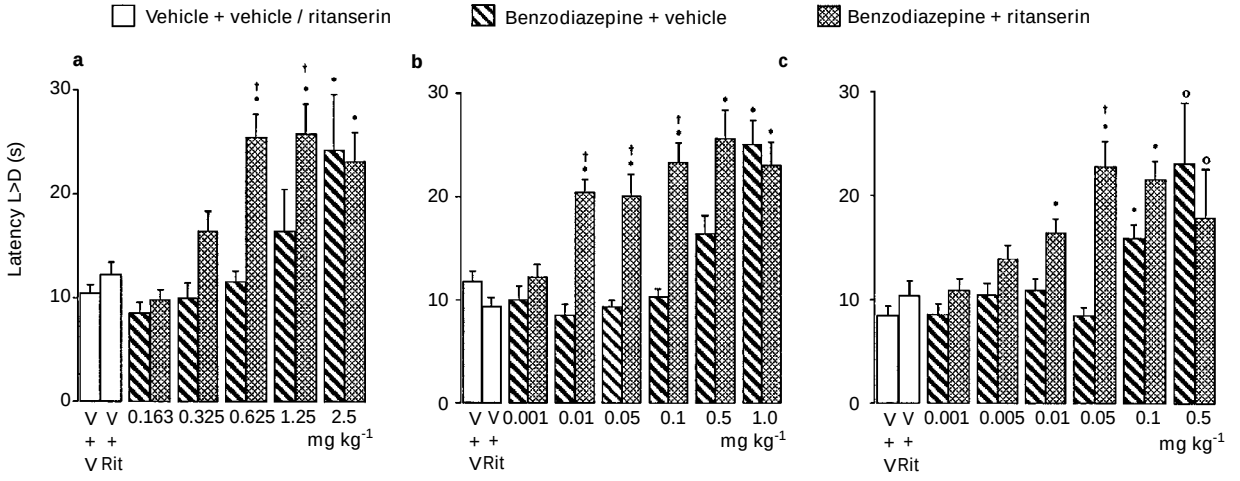

Figure 4 The ability of ritanserin (Rit, 1.0 mg kg^{-1}) to enhance the disinhibitory potency of benzodiazepines, (a) chlordiazepoxide, (b) temazepam and (c) lorazepam, in the mouse light/dark test. Animals received an intraperitoneal injection of vehicle (V) + vehicle, vehicle + ritanserin, benzodiazepine + vehicle or ritanserin. Animals received vehicle or drugs as 40 min pretreatments with testing 40 min after the last administration. Data are presented as the latency of first movement from the light to the dark compartment during the 5 min test period. Each value is the mean ± s.e.mean, $n = 10$. A significant increase in responding compared with V + V is shown as $^*P < 0.01$; a significant increase in responding in the benzodiazepine + ritanserin treated group compared to benzodiazepine + vehicle at any one dose level is shown as $\dagger P < 0.01$ (ANOVA followed by Dunnett's t test). (° Indicates sedation).

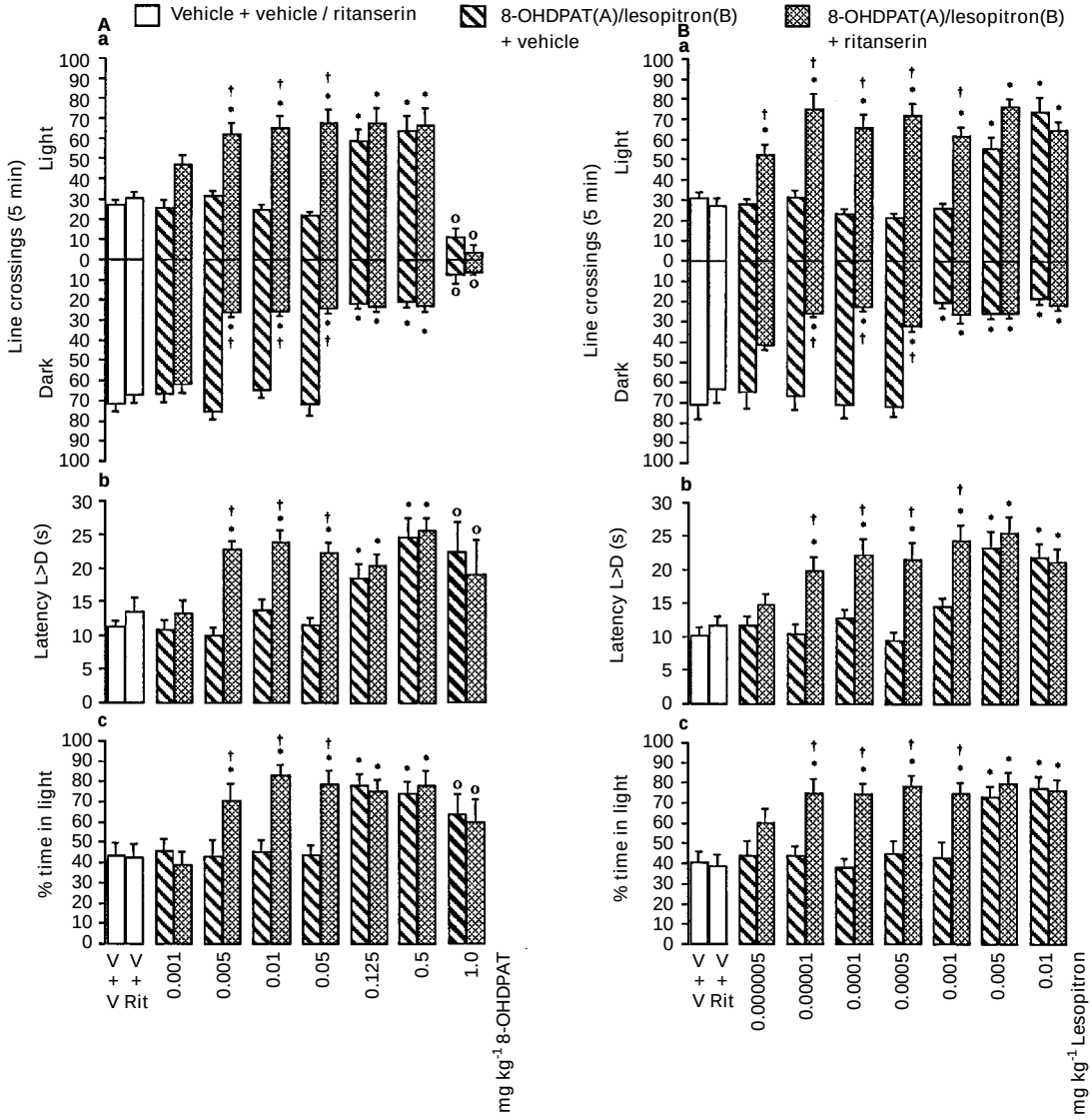

Figure 5 The ability of ritanserin (Rit. 1.0 mg kg^{-1}) to enhance the disinhibitory potency of 8-OH-DPAT and lesopitron in the mouse light/dark test. Animals received an intraperitoneal injection of vehicle + vehicle (V), vehicle + ritanserin, 8-OH-DPAT (A) or lesopitron (B) + vehicle or ritanserin. Vehicle or drugs were administered as 40 min pretreatments with testing 40 min after the last treatment. Data are presented as the latency of first movement from the light to the dark compartment (b), the % time spent in the light area (c) and line crossings in both compartments (a) during the 5 min test period. Each value is the mean ± s.e.mean, $n = 10$. A significant increase or decrease in responding compared with V + V is indicated $^{*}P < 0.01$; a significant increase or decrease in response to the 8-OH-DPAT or lesopitron/ritanserin interaction compared to the 8-OH-DPAT or lesopitron/vehicle treatment at any one dose level is shown as $\dagger P < 0.01$ (ANOVA followed by Dunnett's t test). (° Indicates sedation).

was indistinguishable. There were significant increases in the latency of the first movement from the light to the dark compartment when the higher doses of the drug were used in both regimens. There was also a clear trend for such doses to increase the time spent in the light compartment, although the slightly higher than normal control basal values ensured that such differences did not achieve significance. The angiotensin converting enzyme inhibitor ceranapril (0.0005 – 0.1 mg kg^{-1}) also increased the latency of first movement from the light area and the time spent in this section of the test box. Ceranapril had a comparable action when administered alone or after pretreatment with ritanserin, with only the latency measurement at 0.001 mg kg^{-1} ceranapril being significantly enhanced in the ritanserin pretreated group (Figure 8).

Parachlorophenylalanine (PCPA) Three groups of animals with appropriate vehicle controls received a daily injection of PCPA (200 mg kg^{-1}, i.p.) for 1, 2 or 3 days with behavioural testing 24 h after the last PCPA treatment and 40 min after receiving a single injection of ritanserin (1.0 mg kg^{-1}) or vehicle.

Twenty four hours following a 3 day treatment with PCPA, mice showed an increased latency of movement from the light to the dark area, and increased time spent in the light area. Measurements made following the 1 and 2 day treatments with PCPA were indistinguishable from vehicle-treated controls. However, animals receiving ritanserin following 1 or 2 days PCPA treatment showed a disinhibitory profile, with an increase in the latency measurement and in the time spent in the light area of the test box. The disinhibitory profile normally observed after a 3 day treatment with PCPA alone was not modified by ritanserin administration (Figure 9).

The interaction of GR113808 with disinhibitory agents in the mouse light/dark test

Five treatment groups were used to investigate the interaction between the disinhibitory agents with GR113808: (i) vehi-

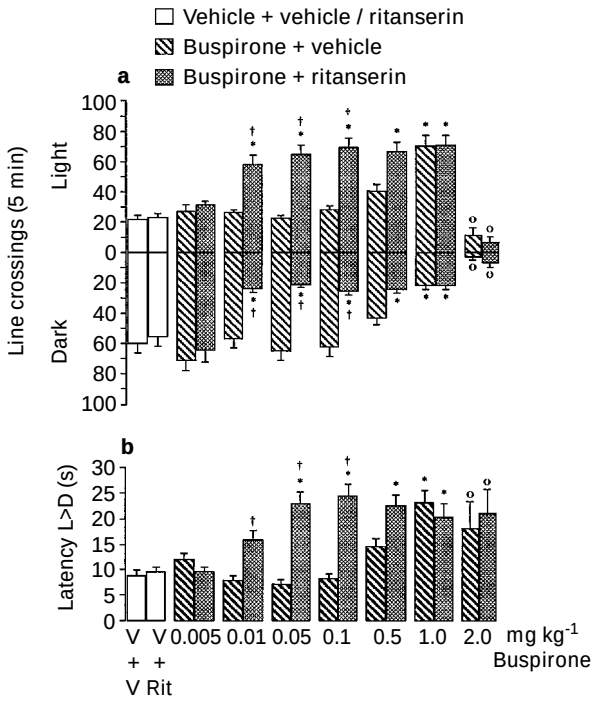

Figure 6 The ability of ritanserin (Rit., 1.0 mg kg^{-1}) to enhance the disinhibitory potency of buspirone in the mouse light/dark test. Animals received an intraperitoneal injection of vehicle + vehicle (V), vehicle + ritanserin, buspirone + vehicle or ritanserin. Vehicle or drugs were administered as 40 min pretreatments with testing 40 min after the last treatment. Data are presented as the latency of first movement from the light to the dark compartment (b) and line crossings in both compartments (a) during the 5 min test period. Each value is the mean ± s.e.mean, $n=10$. A significant increase or decrease in responding compared with V + V is indicated $^{*}P<0.01$; a significant increase or decrease in response to the buspirone/ritanserin interaction compared to the buspirone/vehicle treatment at any one dose level is shown as $\dagger P<0.01$ (ANOVA followed by Dunnett's t test). (° Indicates sedation).

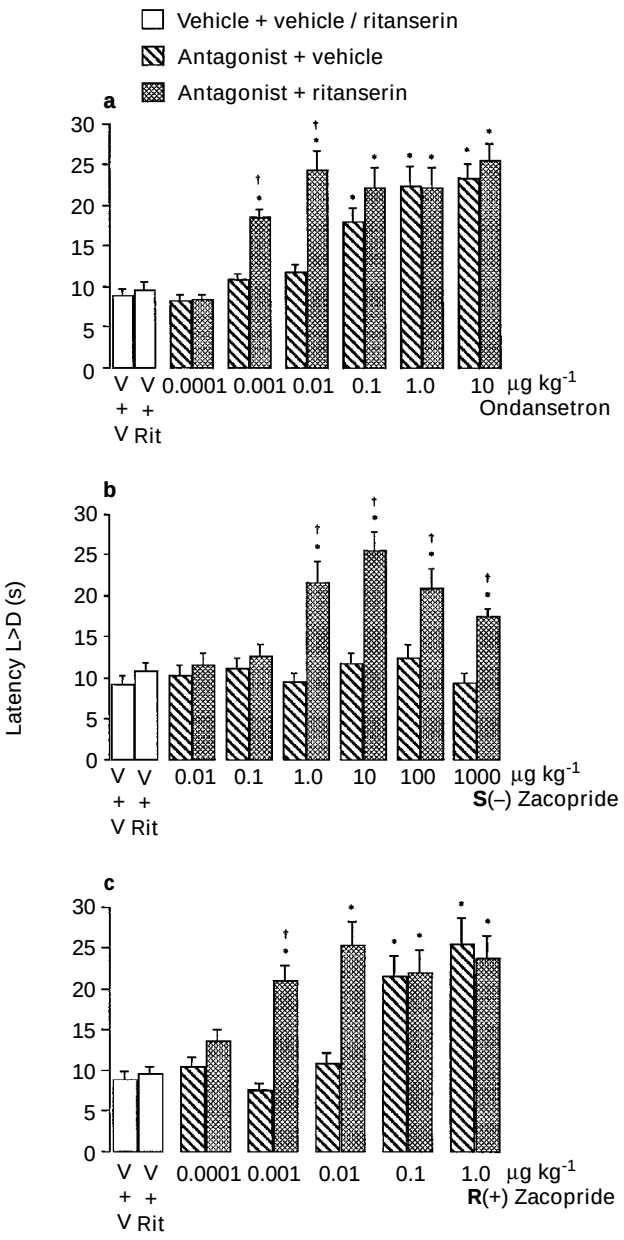

Figure 7 The ability of ritanserin (Rit., 1.0 mg kg^{-1}) to enhance the disinhibitory potency of 5-HT_3 receptor antagonists (a) ondansetron, (b) **S**(−)-zacopride and (c) **R**(+)-zacopride, in the mouse light/dark test. Animals received an intraperitoneal injection of vehicle + vehicle (V), vehicle + ritanserin, 5-HT_3 receptor antagonist + vehicle or ritanserin. Vehicle or drugs were administered as 40 min pretreatments with testing 40 min after the last treatment. Data are presented as the latency of first movement from the light to the dark compartment during the 5 min test period. Each value is the mean ± s.e.mean, $n=10$. A significant increase in responding compared with V + V is indicated $^{*}P<0.01$; a significant increase in response to the 5-HT_3 receptor antagonist/ritanserin interaction compared to the 5-HT_3 receptor antagonist/vehicle treatment at any one dose level is shown as $^{+}P<0.01$ (ANOVA followed by Dunnett's t test).

cle + vehicle, (ii) disinhibitory agent + vehicle or (iii) GR113808 (0.0001 mg kg^{-1}), (iv) GR113808 (0.001 mg kg^{-1}) and (v) GR113808 (0.01 mg kg^{-1}). The experiments were then repeated. The doses of GR113808 (0.0001, 0.001 and 0.01 mg kg^{-1}) were determined from preliminary experiments as the range required to antagonize the disinhibitory effects of the benzodiazepines and other agents. Preliminary experiments also established that the administration of the three doses of GR113808 alone failed to modify mouse behaviour in the light/dark test. Therefore, these three doses of GR113808 administered alone were not routinely included in every test session but, with a vehicle control and mice in group sizes of 5, were administered 4 times throughout the period of experimentation to ensure their continued inactivity. Analysis of the 4 sets of data indicated that there was no significant effect of treatment or session, ($F_{(3,16)}$ ratios were in the range of 0.4–1.02 with P values of 0.41–0.72), with collapsed mean data for latency values being: for vehicle 10.25 ± 0.48 s, GR113808 (0.0001 mg kg^{-1}) 11.2 ± 0.5 s, GR113808 (0.001 mg kg^{-1}) 9.35 ± 0.4 s and GR113808 (0.01 mg kg^{-1}) 11.3 ± 0.59 s ($F_{(3,76)}=3.39$, $P=0.023$).

An analysis of the 14 data sets shown in Tables 1 and 2 indicated that vehicle control responses remained consistent throughout the experiments ($F_{13,126}=0.97$, $P=0.48$ and $F_{13,126}=1.3$, $P=0.55$ for values of latency and % time in light area, respectively) with a mean value for all experiments of 10.5 ± 0.3 s and 44.2 ± 1.3% for the latency values and % time spent in the light area, respectively. The doses of disinhibitory agents were selected from the previous studies as those causing a maximal disinhibitory effect. The intensity of response to chlordiazepoxide and the other agents alone were in the range of 16.7 ± 1.37 to 23.7 ± 2.4 s for latency and 71.1 ± 6.9 to 79.7 ± 6.8% for time spent in the light area and were of indistinguishable intensity with respect to latency measurements ($F_{13,126}=1.37$, $P=0.178$) and to the % time spent in the light area ($F_{13,126}=0.13$, $P=0.99$): the mean latency and % time spent in the light area values for all the 14 treatments were, respectively, 21.2 ± 0.7 s and 74.8 ± 1.7%. The above data ensure a comparable baseline performance

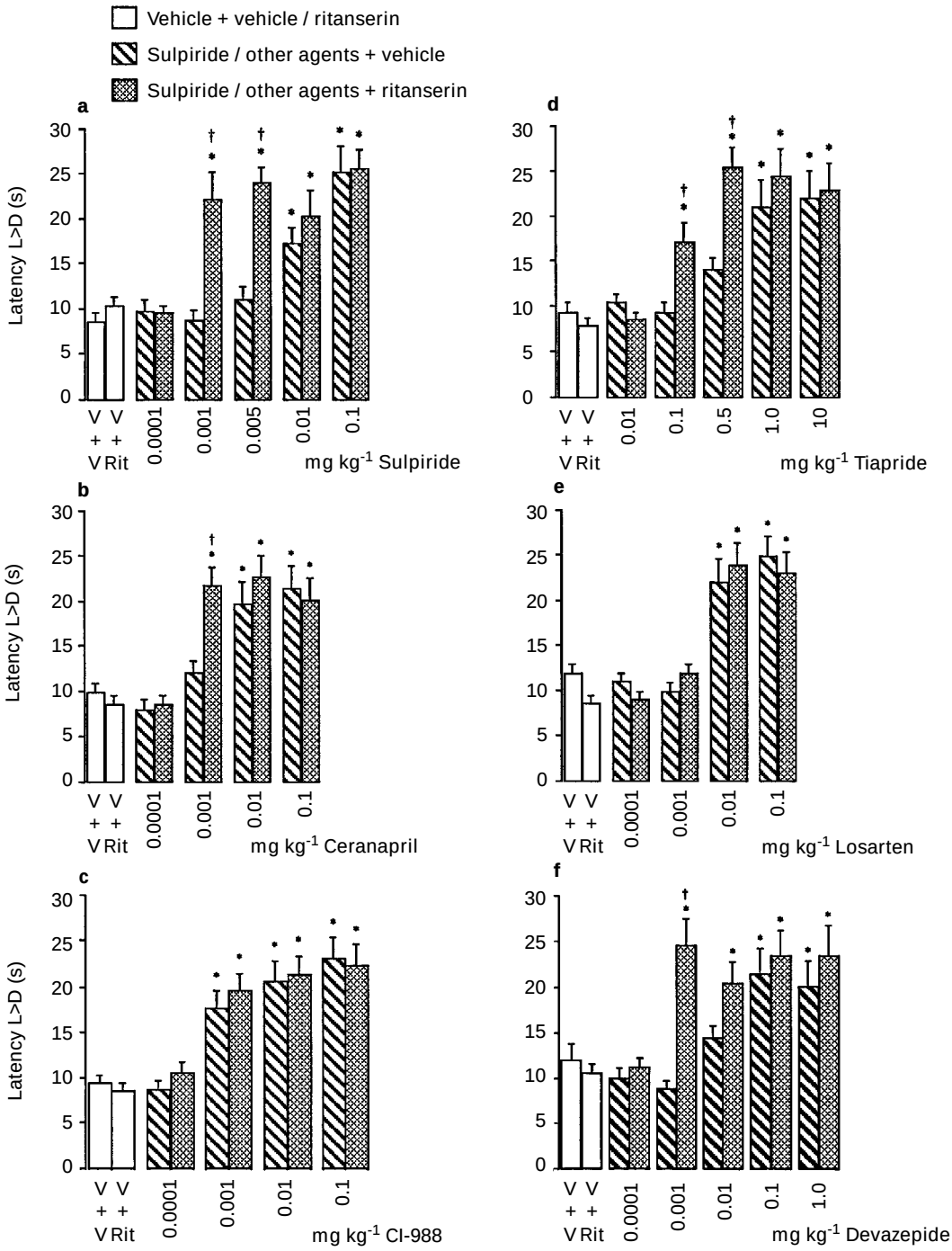

Figure 8 The ability of ritanserin (Rit., 1.0 mg kg^{-1}) to enhance the disinhibitory potency of (a) sulpiride and other agents (b, ceranapril; c, CI-988; d, tiapride; e, losarten; f, devazepide) in the mouse light/dark test. Animals received an intraperitoneal injection of vehicle + vehicle (V), vehicle + ritanserin, sulpiride or other agent + vehicle or ritanserin. Vehicle or drugs were administered as 40 min pretreatments with testing 40 min after the last treatment. Data are represented as the latency of first movement from the light to the dark compartment during the 5 min test period. Each value is the mean ± s.e.mean, $n = 10$. A significant increase in responding compared with V + V is indicated by $*P < 0.01$; a significant increase in response to the sulpiride or other agents/ritanserin interaction compared to the sulpiride or other agents/vehicle treatment at any one dose level is shown as $\dagger P < 0.01$ (ANOVA followed by Dunnett's t test).

between experiments and a comparable intensity of disinhibitory change induced by chlordiazepoxide and the other treatments.

The disinhibitory profiles of action of chlordiazepoxide (2.5 mg kg^{-1}), lorazepam (0.1 mg kg^{-1}), 8-OH-DPAT (0.5 mg kg^{-1}), buspirone (1.0 mg kg^{-1}), lesopitron (0.01 mg kg^{-1}), ondansetron (0.01 mg kg^{-1}), **R**(+)-zacopride (0.001 mg kg^{-1}), sulpiride (5.0 mg kg^{-1}), tiapride (5.0 mg kg^{-1}), devazepide (0.1 mg kg^{-1}), losarten (0.1 mg kg^{-1}), ceranapril (0.1 mg kg^{-1}), CI-988 (0.001 mg kg^{-1}) and PCPA (200 mg kg^{-1} daily for 3 days) were similarly influenced by the dosage regimens of GR113808. Thus, the low dose of GR113808 (0.0001 mg kg^{-1}) failed to modify the disinhibitory profile of any drug treatment. The intermediate dose of GR113808 0.001 mg kg^{-1} characteristically reduced the disinhibitory effect of the drug treatments but this did not usually achieve significance. However, antagonism of the disinhibitory profiles of all 14 treatments achieved significance with GR113808 at 0.01 mg kg^{-1}, the values returning to those of the vehicle treated controls (Tables 1 and 2).

On no occasion was the interaction between GR113808 and any of the disinhibitory treatments associated with a reduction in either latency or the % time spent in the light area to values significantly below vehicle-treated controls.

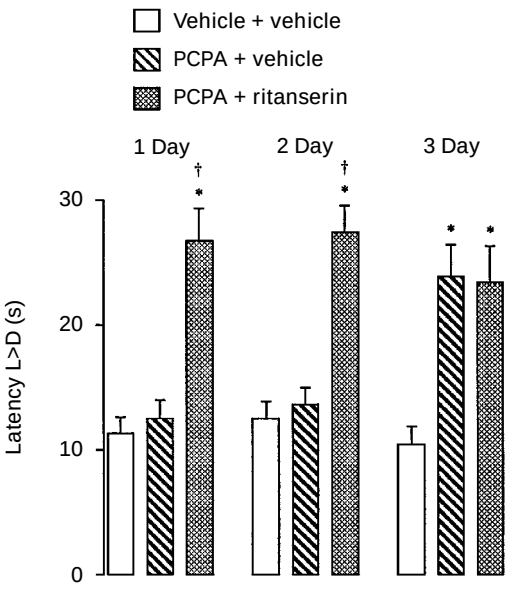

Figure 9 The ability of ritanserin to enable the appearance of the disinhibitory effect of parachlorophenylalanine (PCPA) in the mouse light/dark test. Animals received an intraperitoneal injection of PCPA (200 mg kg^{-1}) or vehicle (V) for 1, 2 or 3 days followed by behavioural testing 24 h following the last PCPA injection and 40 min after a vehicle or ritanserin (Rit., 1.0 mg kg^{-1}) treatment. Data are presented as the latency of first movement from the light to the dark compartment during the 5 min test period. Each value is the mean ± s.e.mean of 10 animals. A significant increase in responding to V + V is shown as $*P<0.001$; a significant increase in responding compared to PCPA + V on each day of testing is shown as $\dagger P<0.01$ (ANOVA followed by Dunnett's *t* test).

Discussion

The ability of ritanserin to enhance the disinhibitory potency of diazepam in the mouse light/dark test (Costall & Naylor, 1994a) was confirmed, established to be dose-related, and extended to other antagonists at the 5-HT_2 receptor, RP62203, MDL11939 and methysergide (Dudley *et al*., 1988; Doble *et al*., 1992; Zifa & Fillion, 1992). Ritanserin was also shown to enhance the disinhibitory potency of other benzodiazepines, chloridazepoxide, temazepam and lorazepam, the 5-HT_{1A} receptor ligands 8-OH-DPAT, busipirone and lesopitron (Hjorth & Carlsson, 1982; Hjorth *et al*., 1982; Costall *et al*., 1992), the 5-HT_3 receptor antagonists, ondansetron and **R**(+)-zacopride (Jones *et al*., 1988; Barnes *et al*., 1992a), the substituted benzamides, sulpiride and tiapride (Barry *et al*., 1987), and the CCK_A receptor antagonist, devazepide (Ravard *et al*., 1990). The increase in potency of the disinhibitory agents varied considerably. Thus, the benzodiazepines showed no more than a 10 fold increase, whilst there was a 25, 100 and 500 fold increase in potency to 8-OH-DPAT, buspirone and lesopitron respectively. The potency of ondansetron and R(+)-zacopride was enhanced 5 to 10 fold. Even more dramatically, **S**(−)-zacopride when administered alone caused no behavioural change. But when combined with ritanserin it was as efficacious as diazepam or any of the other treatments at disinhibiting behaviour, revealing a potency increase of at least 1000 fold.

The magnitude of the behavioural changes and the diverse chemical structures of the many compounds examined suggest that potential changes in drug metabolism are unlikely to contribute importantly to the synergisms observed. Also, ritanserin exerted a selective increase in drug-induced disinhibitory potency since it failed to modify the disinhibitory effects of the angiotensin AT_1 receptor antagonist losarten (Polidori *et al*., 1996), the angiotensin converting enzyme inhibitor ceranapril (Cushman *et al*., 1989) or the selective CCK_B receptor antagonist CI-988 (Hughes *et al*., 1990). It is interesting that whilst the disinhibitory effects of the CCK receptor antagonists parallel their affinity for the CCK_B rather than the CCK_A receptor (Singh *et al*., 1995), ritanserin appears to distinguish between the CCK_A and CCK_B receptor antagonists in the

Table 1 The ability of GR113808 (GR) to antagonize the disinhibitory effects of chlordiazepoxide (c) and other agents in the mouse light/dark test

Treatment	*Latency (s)*	*% time in light*	*Treatment*	*Latency (s)*	*% time in light*
Vehicle	11.7 ± 0.01	41.4 ± 5.1	Vehicle	11.5 ± 1.4	44.4 ± 5.2
Chlordiazepoxide (C,2.5 mg kg^{-1})	23.2 ± 2.6*	76.7 ± 5.6*	Lesopitron (L, 10 μg kg^{-1})	21.9 ± 2.3*	72.7 ± 5.7*
C + GR 0.0001 mg kg^{-1}	21.7 ± 2.5*	77.6 ± 5.6*	L + GR 0.0001 mg kg^{-1}	18.0 ± 2.1	69.4 ± 5.2*
C + GR 0.001 mg kg^{-1}	12.6 ± 1.2$^+$	50.8 ± 4.9$^+$	L + GR 0.001 mg kg^{-1}	14.6 ± 1.3	65.8 ± 7.1
C + GR 0.01 mg kg^{-1}	10.2 ± 1.0$^+$	44.8 ± 4.8$^+$	L + GR 0.01 mg kg^{-1}	9.4 ± 1.2$^+$	49.4 ± 3.3$^+$
Vehicle	9.7 ± 1.18	48.9 ± 4.9	Vehicle	12.0 ± 1.0	41.6 ± 6.2
Lorazepam (L, 0.1 mg kg^{-1})	23.7 ± 2.4*	75.1 ± 6.9*	Ondansetron (ON, 10 μg kg^{-1})	23.6 ± 1.9*	72.3 ± 7.7*
L + GR 0.0001 mg kg^{-1}	22.0 ± 1.9*	71.6 ± 6.2	ON + GR 0.0001 mg kg^{-1}	21.1 ± 2.6*	70.0 ± 8.7*
L + GR 0.001 mg kg^{-1}	18.7 ± 1.9	56 ± 5.1	ON + GR 0.001 mg kg^{-1}	16.8 ± 1.6	64.0 ± 6.0
L + GR 0.01 mg kg^{-1}	14.1 ± 0.99$^+$	53.6 ± 4.9	ON + GR 0.01 mg kg^{-1}	11.5 ± 1.1$^+$	45.5 ± 4.3$^+$
Vehicle	9.2 ± 1.5	48.2 ± 6.2	Vehicle	10.3 ± 0.66	43.3 ± 5.1
8-OH-DPAT (DP, 0.5 mg kg^{-1})	18.7 ± 2.2*	73.7 ± 5.6	R(+)zacopride (Z, 1.0 μg kg^{-1})	22.6 ± 1.92*	79.7 ± 6.8*
DP + GR 0.0001 mg kg^{-1}	18.5 ± 2.4*	76.1 ± 5.9	Z + GR 0.0001 mg kg^{-1}	24.2 ± 2.8*	72.5 ± 6.1*
DP + GR 0.001 mg kg^{-1}	14.2 ± 2.1	67.2 ± 7.7	Z + GR 0.001 mg kg^{-1}	16.4 ± 2.0	58.7 ± 6.8
DP + GR 0.01 mg kg^{-1}	8.9 ± 0.9$^+$	44.8 ± 4.7	Z + GR 0.01 mg kg^{-1}	9.0 ± 1.4$^+$	43.9 ± 5.5$^+$
Vehicle	8.8 ± 1.3	47.9 ± 6.26	Vehicle	9.4 ± 1.25	44.6 ± 4.7
Buspirone (B, 1.0 mg kg^{-1})	16.7 ± 1.7*	71.5 ± 7.3	PCPA	17.7 ± 1.6*	74.9 ± 6.2*
B + GR 0.0001 mg kg^{-1}	14.4 ± 2.6	70.5 ± 6.0	PCPA + GR 0.0001 mg kg^{-1}	18.5 ± 2.1*	72.6 ± 5.0*
B + GR 0.001 mg kg^{-1}	15.8 ± 1.2*	63.3 ± 7.4	PCPA + GR 0.001 mg kg^{-1}	16.6 ± 1.7	68.0 ± 5.9
B + GR 0.01 mg kg^{-1}	9.0 ± 1.3$^+$	48.1 ± 5.3	PCPA + GR 0.01 mg kg^{-1}	12.0 ± 1.2$^+$	42.4 ± 4.7$^+$

Values (the mean ± s.e.mean of 10 determinations) are the latency of first movement from the light to the dark compartment and the % time spent in the light compartment. $*P<0.01$ with respect to vehicle control and $^+P<0.01$ with respect to the administration of chlordiazepoxide and other disinhibitory agents alone (one way ANOVA followed by Dunnett's *t* test). Chlordiazepoxide and the other agents were administered i.p. as a 40 min pretreatment to GR113808 with testing after a further 40 min period. Parachlorophenylalinine (PCPA) was administered daily for 3 days (200 mg kg^{-1} i.p.) with testing 24 h after withdrawal from treatment.

Table 2 The ability of GR113808 (GR, 0.0001–0.01 mg kg^{-1}) to antagonize the disinhibitory effects of sulpiride and other agents in the mouse light/dark test

Treatment	*Latency (s)*	*% time in light*	*Treatment*	*Latency (s)*	*% time in light*
Vehicle	10.8±1.25	40.9±3.3	Vehicle	10.3±0.8	45.4±5.1
Sulpiride (S, 0.1 mg kg^{-1})	19.8±2.4*	75.7±7.2*	Losarten (L, 0.1 mg kg^{-1})	23.4±2.0*	76.7±6.9*
S+GR 0.0001 mg kg^{-1}	21.0±2.6*	70.3±5.6*	L+GR 0.0001 mg kg^{-1}	20.6±2.2*	72.5±7.3*
S+GR 0.001 mg kg^{-1}	15.9±1.7	62.2±6.5	L+GR 0.001 mg kg^{-1}	16.3±1.5	63.1±6.2
S+GR 0.01 mg kg^{-1}	12.2±1.2^{+}	43.9±6.3^{+}	L+GR 0.01 mg kg^{-1}	10.7±0.7^{+}	47.2±5.5^{+}
Vehicle	11.2±0.8	41.8±5.2	Vehicle	9.9±1.08	44.4±3.4
Tiapride (T, 5.0 mg kg^{-1})	22.1±2.4*	73.7±6.9*	Ceranapril (C, 0.1 mg kg^{-1})	18.3±1.35*	71.1±6.5*
T+GR 0.0001 mg kg^{-1}	20.6±2.7*	72.3±7.4*	C+GR 0.0001 mg kg^{-1}	20.4±1.8*	74.7±6.0*
T+GR 0.001 mg kg^{-1}	17.3±1.5	66.4±6.1	C+GR 0.001 mg kg^{-1}	14.0±2.0	51.5±3.4
T+GR 0.01 mg kg^{-1}	10.8±1.1^{+}	45.4±5.0^{+}	CC+GR 0.01 mg kg^{-1}	9.9±1.2^{+}	42.8±5.5^{+}
Vehicle	10.4±1.4	42.8±6.3	Vehicle	9.9±1.08	44.2±5.4
Devazepide (D, 0.1 mg kg^{-1})	23.7±1.99*	76.3±6.9*	CI-988 (C, 0.001 mg kg^{-1})	18.3±1.35*	71.1±6.5*
D+GR 0.0001 mg kg^{-1}	21.7±2.5*	75.0±7.7*	C+GR 0.0001 mg kg^{-1}	20.4±1.8*	74.7±6.0*
D+GR 0.001 mg kg^{-1}	12.7±1.6^{+}	53.5±4.9	C+GR 0.001 mg kg^{-1}	14.0±2.0	55.5±4.4
D+GR 0.01 mg kg^{-1}	10.5±1.09^{+}	44.4±5.8^{+}	C+GR 0.01 mg kg^{-1}	9.9±1.2^{+}	42.8±5.5^{+}

Values (the mean±s.e.mean of 10 determinations) are the latency of first movement from the light to the dark compartment and the % time spent in the light compartment. *$P<0.01$ with respect to vehicle control and $^{+}P<0.01$ with respect to the administration of sulpiride and other disinhibitory agents alone (one way ANOVA followed by Dunnett's t test). Sulpiride and the other agents were administered as a 40 min pretreatment to GR113808 with testing after a further 40 min period.

present study, enhancing the potency of the CCK_A receptor antagonist devazepide but not that of CI-988.

The ability of the 5-HT_2 receptor antagonists to enhance or reveal a drug-induced disinhibitory profile in the mouse test is interpreted as a pharmacological interaction which may occur in a number of ways. But central to these interpretations are the known actions of 5-HT_2 receptor antagonists to attenuate the inhibitory effects of 5-hydroxytryptophan and other 5-HT receptor agonists in the rodent (Kennett *et al*., 1989; Cheng *et al*., 1994; Costall & Naylor, 1995). Indeed, the administration of a 5-HT_2 receptor antagonist alone in some paradigms may be sufficient to cause a disinhibitory effect (Kennett, 1992; Kennett *et al*., 1994), although this did not occur in the present or other experiments (Costall & Naylor, 1995). Nevertheless, the antagonism of a 5-HT behavioural inhibitory mechanism by the 5-HT_2 receptor antagonists may synergize with subthreshold doses of diazepam and the other agents to precipitate a disinhibitory or anxiolytic profile. Alternatively, diazepam and the other agents may be considered to be revealing a disinhibitory potential of the 5-HT_2 receptor antagonists. But in either event, the synergism could occur as a result of the 5-HT_2 receptor antagonists and the disinhibitory agents acting on different systems, the consequences of the separate actions being recognised and mediated at a subsequent site. However, the reason for the degrees of enhancement in potency varying from approximately 10 with the benzodiazepines to 500 with lesopitron is not clear. Perhaps the greater enhancement of potency with lesopitron and other 5-HT_{1A} receptor ligands might result from their agonist action on somatodendritic 5-HT_{1A} receptors in the raphe nuclei to reduce 5-HT cell firing and 5-HT release in the forebrain (Sharp *et al*., 1989; Blier *et al*., 1993; Routledge *et al*., 1995). A reduction in 5-HT synthesis and release by PCPA may also account for the more rapid development of a disinhibitory profile when used with ritanserin. The effects of PCPA and the 5-HT_{1A} receptor ligands to reduce 5-HT release could be readily hypothesized to enhance the effects of a postsynaptic 5-HT_2 receptor blockade, irrespective of effects on other transmitter systems.

It remains puzzling that the behavioural effects of lesopitron are achieved at doses lower than those reducing neuronal firing in the dorsal raphe nucleus or 5-HT release in the forebrain, and that the effect of lesopitron to inhibit cell firing lasts for only a few minutes (Haj-Dahmane *et al*., 1994). Furthermore, a major metabolite of lesopitron (E5043) is also active at disinhibiting behaviour in rodent models of anxiety but has no affinity for the 5-HT_{1A} receptor (see Haj-Dahmane *et al*., 1994). This cautions against an interpretation of drug effect as a sole action of the parent compound or as an exclusive action on the 5-HT_{1A} receptor.

The 5-HT_3 receptor antagonists ondansetron and **R**(+)-zacopride also disinhibit behavioural responding to aversive situations in the rodent and primate (Jones *et al*., 1988; Barnes *et al*., 1992a), attenuate the inhibitory effects of 5-hydroxytryptophan in the mouse and rat (Cheng *et al*., 1994) and the 5-HT_3 receptor agonist m-chlorophenylbiguanide in the mouse light/dark test (Costall *et al*., unpublished data). In the present study ritanserin enhanced the potency of odansetron and **R**(+)-zacopride 100 fold, indicating a synergistic interaction between 5-HT_2 and 5-HT_3 receptor blockade. The substituted benzamide derivative sulpiride is a dopamine D_2 receptor antagonist and has no known actions to reduce 5-HT function; the ability of ritanserin to enhance its disinhibitory potency 10 fold remains to be explained. However, dopamine afferents facilitate the release of 5-HT in the raphé dorsalis and inhibit its release in the striatum (Lee & Geyer, 1984; Ferré & Artigas, 1993; Ferré *et al*., 1994). The acute administration of sulpiride and tiapride to antagonize such effects may indirectly influence the 5-hydroxytryptaminergic system. This is to be distinguished from the chronic administration of dopamine receptor antagonists which may interfere with the genomic actions of dopamine receptor stimulation to inhibit the expression of 5-HT_{2A} receptors (Laprade *et al*., 1996).

A quite different explanation of the synergistic interaction between the 5-HT_2 receptor antagonists and diazepam and the other disinhibitory agents may relate to a plurality of actions of the latter agents to both inhibit (via the 5-HT_2 system) and disinhibit behaviour via other systems. Thus, the balanced inhibitory and disinhibitory actions in the presence of ritanserin would be to 'reveal' the disinhibitory potential. However, for two reasons, this is unlikely to occur. Firstly, none of the disinhibitory agents tested have agonist action or affinity for the 5-HT_2 receptor, inhibit 5-HT reuptake processes or cause the release of endogenous 5-HT, i.e. they have no pre- or postsynaptic effects to enhance 5-HT function. Secondly, even when the disinhibitory effects of diazepam and the other agents were blocked by 5-HT_4 receptor antagonism, the disinhibitory effects were never reversed to inhibition (see below). This aruges strongly against a potential role for diazepam and the other compounds actually to inhibit behaviour. However, it is emphasized that such conclusions are based on acute drug administrations and may not apply to the actions of ritanserin to reveal inhibitory effects following withdrawal from repeated treatment with diazepam (Hitchcott *et al*., 1990; Costall & Naylor, 1994a). Furthermore, from the present study with the 5-HT_4 receptor antagonists, evidence was obtained to indicate that all the disinhibitory agents examined mediated their effects via a 5-HT_4 receptor system.

Preliminary evidence for a role of 5-HT_4 receptors to moderate behavioural responding to aversive situations came from the ability of the mixed 5-HT_3/5-HT_4 receptor antagonists SDZ205-557 and tropisetron (but not ondansetron) to antagonize the disinhibitory effects of a 5-hydroxytryptophan/ritanserin regimen, diazepam and 8-OH-DPAT in the rodent (Costall *et al*., 1993a; Cheng *et al*., 1994). In the present study the antagonistic action of SDZ205-557 against diazepam was shown to be dose-related and was extended to the highly specific and selective 5-HT_4 receptor antagonists GR113808 (Gale *et al*., 1994) and SB204070 (Wardle *et al*., 1993). GR113808 was also shown to cause dose-related reductions in the disinhibitory effects of chlordiazepoxide, lorazepam, 8-OH-DPAT, buspirone, lesopitron, ondansetron, **R**(+)-zacopride, sulpiride, tiapride, CI-988, devazepide, losarten and ceranapril. Each of the treatments proved equally sensitive to the antagonistic effects of GR113808; the data offer substantial support for an involvement of 5-HT_4 receptors in the mediation of drug-induced disinhibitory behaviour.

The initial paradox of the present findings is that agents acting to reduce 5-HT function by postsynaptic 5-HT receptor blockade (e.g. ondansetron) or by a reduction of 5-HT synthesis, release or cell firing (e.g. buspirone, diazepam, **R**(+)-zacopride) (see Introduction) should have their behavioural effects attenuated by a further 5-HT receptor blockade afforded by GR113808. However, this would occur if the consequences of the effect of the benzodiazepines, 5-HT_3 or 5-HT_{1A} receptor ligands to reduce 5-HT function is to remove an excitatory input to a neuronal mechanism normally inhibiting the 5-HT cell mediating its effects via a 5-HT_4 receptor system. In effect, removal of the inhibitory input would act to facilitate 5-HT function at the 5-HT_4 site. Evidence that the 5-HT_4 site may mediate an anxiolytic profile is obtained from the use of the 5-HT_4 agonist Z-019(N-[1-(3-(tetrahydrofuryl)propyl]-4-piperidyl]-2-propyloxy-4-amine-5-chlorbenzamide), which is at least a 100 times as potent as diazepam at disinhibiting behaviour in the mouse light/dark test and rat social interaction (Fernandez *et al*., 1994). Also, the anxiolytic-like actions of 5-hydroxytryptophan revealed by ritanserin in the mouse light/dark test and rat social interaction are antagonized by SDZ205-557 (Costall *et al*., 1993a,b) and GR113808 (Costall, unpublished data).

However, a broader role for the 5-HT_4 receptor in mediating disinhibitory behaviour comes from the antagonistic effect of GR113808 on the disinhibitory profile of the CCK_A and CCK_B receptor antagonists devazepide and CI-988 (Hughes *et al*., 1990; Ravard *et al*., 1990), the angiotensin AT_1 receptor antagonist losarten (Barnes *et al*., 1990), the angiotensin converting enzyme inhibitor ceranapril (Costall *et al*., 1990) and the substituted benzamides sulpiride and tiapride (Barry *et al*., 1987). It is not known how treatments which affect the cholecystokinin and angiotensin systems can influence the 5-HT_4 receptor-mediated changes in behaviour. However low concentrations of CCK and caerulein facilitate the K^+-evoked release of γ-aminobutyric acid (GABA) from slices of rat cerebral cortex and this effect is blocked by a CCK_B receptor antagonist (Belleroche & Bandopadhyay, 1992). Caerulein, CCK-8 or CCK_4 can induce panic attacks in man (Abelson & Nesse, 1990; Bradwejn *et al*., 1990) and anxiety-like behaviour in rodents (see Harro *et al*., 1990; Guimarães *et al*., 1992) which are antagonized by CCK receptor antagonists and benzodiazepines, the latter also inhibiting the activation of hippocampal neurones caused by CCK (Bradwejn & De Montigny, 1984). The studies indicate that the mechanisms mediating the effects of CCK and the benzodiazepines may be closely related and a preliminary microdialysis study indicates that CCK may elevate extracellular levels of 5-HT (Isogawa *et al*., 1996). However, whilst sulpiride and tiapride have well established actions as dopamine receptor antagonists, they are not known to interact directly with the 5-hydroxytryptaminergic systems (Jenner & Marsden, 1979; Schwartz *et al*., 1982; Chivers *et al*., 1983).

The ability of GR113808 to antagonize the disinhibitory effects of such agents indicates that such treatments may directly or indirectly enhance 5-HT function. Therefore, a reduced 5-HT neurotransmission would be predicted to attenuate their effects. However, the results from such experiments designed to test this hypothesis are difficult to interpret. Thus, mice show a disinhibition of behaviour in the light/dark test following the administration of devazepide, losarten and ceranapril after pretreatment with parachlorophenylalanine to reduce 5-HT synthesis (Barnes *et al*., 1992a). However, the effect of the parachlorophenylalanine treatment alone was to disinhibit behaviour and this may obscure a change in response to the associated drug treatments. Also, the stores of 5-HT remaining after parachlorophenylalanine treatment may be sufficient to maintain a functional role. This is supported by findings from the present studies which have shown that the disinhibitory profile of parachlorophenylalanine treatment itself is antagonized by GR113808. This is a particularly interesting observation, indicating that a disinhibitory profile observed even in the presence of a reduced 5-HT synthesis can be antagonized by a 5-HT_4 receptor blockade. In turn, this may also indicate that a low level of 5-HT stimulation at the 5-HT_4 receptor may be sufficient to trigger a disinhibitory profile.

The presence of opposing behaviourally inhibitory and disinhibitory 5-HT mechanisms may contribute to an understanding of the controversial data on the varying effects of pharmacological manipulations of 5-HT function. For example, Eglen *et al*. (1994) showed that **S**(−)-zacopride in the C57BL/6 mouse strain had an anxiolytic profile in the mouse light/dark test, whereas in the present study with BKW mice it was ineffective. However, in the presence of ritanserin, the present study demonstrated disinhibitory potencies for both **R**(+)- and **S**(−)-zacopride similar to those found by Eglen *et al*. (1994). Such data may indicate that the C57BL/6 mouse strain either lacks the ritanserin sensitive mechanism or that it operates at a reduced level to that found in the BKW mouse.

Different degrees of 5-HT tone between species or strains of animals on the inhibitory or disinhibitory mechanisms would also create differences in the appearance of agonist or antagonist action. For example, the present failure of ritanserin to modify behaviour when administered alone is in keeping with a general ineffectiveness of 5-HT_2 receptor antagonists to modify physiological processes (Leysen, 1992). Yet Kennett (1992) has shown that 5-HT_2 receptor antagonists have an anxiolytic profile in their own right in the rat social interaction test, indicating the presence of 5-HT tone in this model. This could occur through natural predisposition or could be a consequence of the manner of handling of animals during use (see Brett & Pratt, 1990; File *et al*., 1992; Andrews & File, 1993). A raised 5-HT tone may also help to explain the enhanced anxiolytic effect of 5-HT_2 receptor antagonists or the isomers of zacopride in diazepam or alcohol withdrawn rats (Hitchcott *et al*., 1990; File & Andrews, 1993; Lal *et al*., 1993).

5-HT_2 and 5-HT_4 receptors are widely distributed within the brain (Radja *et al*., 1991; Grossman *et al*., 1993; Waeber *et al*., 1994) and future studies are required to determine the precise sites of action of 5-HT_2 and 5-HT_4 receptor antagonists to modify behavioural responding to aversive situations. The actions of ritanserin, RP62203, MDL11939 and other 5-HT_2 receptor antagonists have generally been attributed to an interaction with the 5-HT_{2A} or 5-HT_{2C} receptors (Dudley *et al*., 1988; Doble *et al*., 1992; Zifa & Fillon, 1992). However, the reassessment of the 5-HT_2 receptor subtype to accommodate the recently cloned 5-HT_{2B} receptor (Hoyer *et al*., 1994), the mRNA transcript being present in the mouse brain (Schmuck *et al*., 1994), provides a further site of action to moderate anxiety-like responding (Kennett *et al*., 1994). Ritanserin has comparable affinity for all three 5-HT_2 receptor subtypes (see review by Baxter *et al*., 1995) and more selective ligands will be required to delineate the role of the 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors in behavioural responding. However, ritanserin and 8-OH-DPAT also have nanomolar affinity for the 5-

HT_7 receptor cloned from the mouse and other species (see review by Lucas & Hen, 1995), and expressed mainly in the brain. It has been suggested that these receptors play a role in circadian rhythms (Lovenberg *et al*., 1993); 8-OH-DPAT produces phase shifts of locomotor activity (Prossor *et al*., 1990) and the depletion of brain 5-HT modifies rodent and hamster circardian rhythms in response to light (Honma *et al*., 1979; Morin & Blanchard, 1991). Whether such changes are relevant to the acute behavioural effects of the ritanserin/drug interactions remains to be established.

The 5-HT_4 receptor gene has been cloned from rat brain and two different cDNAs have been isolated, generating a short and long isoform (Gerald *et al*., 1995). Both isoforms display a high affinity for GR113808 and comparable displacement profiles for zacopride and other 5-HT receptor ligands. Interestingly, such compounds exhibited different potencies in studies of receptor-effector coupling (Gerald *et al*., 1995). Again, the relevance of drug action at these distinctive sites to anxiety-like responding will require the development of selective ligands.

However, for future therapeutic opportunities in the treatment of anxiety, the design of compounds with mixed 5-HT_{1A} and 5-HT_2 receptor profiles is receiving much attention (see review by Barrett & Vanover, 1993; Albinsson *et al*., 1994; Griebel *et al*., 1996). In the present study the synergy afforded by drug interactions at 5-HT_2 and other 5-HT receptors is supportive of such designs. Roles have been identified for both the 5-HT_2 and 5-HT_4 receptors in moderating drug-induced changes in BKW mouse behavioural responding to the aversive situation of the light/dark test. The data indicate that 5-HT may have a complex role in the modification of anxiety-like behaviour in the rodent which may involve both an inhibitory and a disinhibitory potential.

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