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Effects of 5-HT_{1A} receptor ligands in a modified Geller-Seifter conflict model in the rat

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Abstract

In a modified Geller-Seifter conflict procedure, rats were trained to lever-press for food under a multiple variable interval-fixed ratio (VI30: food; FR10: food + shock) schedule of reinforcement. The ability to antagonize response suppression in the punished period is considered a good predictor for anxiolytic activity. Chlordiazepoxide and alprazolam increased punished responding. The 5-HT_{1A} receptor agonists flesinoxan (*R*(+)-*N*-[2[4-(2,3-dihydro-2-2-hydroxymethyl-1,4-benzodioxin-5-yl)-1-piperazinyl]ethyl]-4-fluorobenzoamide; 0.1–10.0 mg/kg) and 8-OH-DPAT (8-hydroxy-2-(di-*n*-propyl-amino)tetralin; 0.03–0.5 mg/kg) significantly increased punished responding, supporting a role of the 5-HT_{1A} receptor in anxiety. 8-OH-DPAT and flesinoxan also reduced unpunished responding. The anxiolytic effects of 8-OH-DPAT and flesinoxan could only be antagonized with a high dose (1.0 and 3.0 mg/kg respectively) of the 5-HT_{1A} receptor antagonist WAY-100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl) cyclohexanecarboxamide trihydrochloride). All doses of WAY-100635 antagonized the 5-HT_{1A}-induced effects on unpunished responding. The dissimilarity in dose-response curve of WAY-100635 on punished and unpunished behaviour poses questions about the mediation of these effects. © 1997 Elsevier Science B.V.

Keywords: 5-HT_{1A} receptor; 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin); Flesinoxan; WAY-100635; Anxiety; Geller-Seifter, conflict; (Rat)

1. Introduction

Drugs affecting 5-HT (5-hydroxytryptamine, serotonin) receptor subtypes have recently been investigated as possible alternatives to benzodiazepine anxiolytics. The partial 5-HT_{1A} receptor agonist buspirone is used in generalized anxiety disorder (Goa and Ward, 1986; Taylor et al., 1985), and preliminary studies have shown that the recently developed phenylpiperazine derivative flesinoxan (*R*(+)-*N*-[2[4-(2,3-dihydro-2-2-hydroxymethyl-1,4-benzodioxin-5-yl)-1-piperazinyl]ethyl]-4-fluorobenzoamide) has anxiolytic and antidepressant properties in humans (Ansseau et al., 1993; Bradford, 1993; Grof et al., 1993). Receptor binding studies have shown that flesinoxan binds with high affinity to 5-HT_{1A} receptors ($K_i = 1.7$ nM; Schipper et al., 1991). It also has affinity for dopamine D_3 ($K_i = 40$ nM; Tulp, personal communication), dopamine D_2 ($K_i = 140$ nM), and 5-HT_{1D} receptors ($K_i = 160$ nM; Schipper et al., 1991). Flesinoxan is a full agonist at the 5-HT_{1A} receptor in vitro and in vivo (Schipper et al., 1991). In drug discrimination studies it completely generalizes to the selective 5-HT_{1A} receptor agonist 8-OH-DPAT (8-hydroxy-2-(di-*n*-propyl-amino)tetralin) (Ybema et al., 1990). Flesinoxan appears effective in animal models based on unconditioned and classical conditioned responses predictive for anxiolytic activity, such as shock-prod burying (Groenink et al., 1995), ultrasonic vocalization and stress-induced hyperthermia (Olivier et al., 1994; Schipper et al., 1991; Zethof et al., 1995).

The main objective of the present study was to investigate the anxiolytic effect of the 5-HT_{1A} receptor agonists 8-OH-DPAT and flesinoxan in an operant animal model for anxiety, the Geller-Seifter conflict procedure. In this

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model release of punished responding is considered a good predictor of clinical anxiolytic activity, and the 'classical' anxiolytics (benzodiazepines and barbiturates) are easily detected in such paradigms (Cook and Davidson, 1973; Geller and Seifter, 1960). In pigeons, the 5-HT_{1A} receptor agonists buspirone (Barrett et al., 1986; Mansbach et al., 1988), 8-OH-DPAT (Mansbach et al., 1988) and flesinoxan (Barrett et al., 1989) reliably show anxiolytic activity. In rats, 5-HT_{1A} receptor agonists are not consistently detected (Sanger, 1990). However, Schreiber and De Vry (1993) reported positive findings with 8-OH-DPAT in a modified Geller-Seifter procedure. This procedure consisted of an unpunished component in which lever pressing was reinforced according to a variable interval of 30 s (VI30) schedule of reinforcement, alternating with a shorter punished component in which lever-pressing produced a reward paired to a punishment according to a fixed ratio 10 (FR10) schedule of reinforcement. We used their paradigm in the present study. This procedure was first validated using two benzodiazepines, chlordiazepoxide and alprazolam, for which anxiolytic effects were predicted. Thereafter we tested two full 5-HT_{1A} receptor agonists, 8-OH-DPAT and flesinoxan, on their anxiolytic effects. To determine whether these effects are indeed mediated by the 5-HT_{1A} receptor, we tried to antagonize them with the 5-HT_{1A} receptor antagonist WAY-100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl) cyclohexanecarboxamide trihydrochloride) (Fletcher et al., 1996). Finally, given flesinoxan's second highest affinity for the dopamine D_2/D_3 receptor, the effect of the dopamine D_2/D_3 receptor agonist quinpirole was also investigated.

2. Materials and methods

2.1. Animals

The experiment was carried out on 16 male Wistar rats of an outbred strain (CPB:Wu; GDL, Utrecht, Netherlands), weighing 200–250 g at the beginning of the experiment. Animals were individually housed in standard Macrolon cages, and were kept under a 12-h light-dark cycle (lights on from 7:00 to 19:00 h); room temperature and humidity were held constant at 22 ± 1°C and 55–60% respectively. Rats were maintained at approximately 85% of their free-feeding weight, by restricting their food intake to 15–16 g of standard rodent food (Hope Farms) during the week and 52–54 g on Fridays for the whole weekend. Food was given at least an hour after the session. Tap water was provided ad libitum, except during testing.

2.2. Apparatus

Experiments were conducted in eight standard operant test chambers housed in ventilated and sound-insulated cubicles. The chambers were equipped with two retractable levers, a pellet dispenser which delivered 45 mg pellets (Noyes, NH, USA) in a tray placed between the levers, a house light and three lights located above each lever and the dispenser. Only the left lever and left light were used, leaving the right lever retracted, and the right light and the dispenser light off during the whole experiment. Each chamber was fitted with a grid floor, through which scrambled electric shocks could be delivered by a shock generator. An IBM-PC with a MED interface was used to control session events and to record data. The operant chambers, the shock generator and the interface were provided by Med Associates (East Fairfield, CT, USA).

2.3. Training procedure

2.3.1. Shaping

Rats were given daily training sessions (15 min). The house light was on during these initial sessions. After initial lever response shaping, rats were trained to respond according to a FR10 schedule of reinforcement. Once acquired, the FR10 schedule was gradually replaced by a VI30 schedule, until a stable performance had developed.

2.3.2. Conflict schedule

The conflict procedure was adopted from Schreiber and De Vry (1993). It consisted of a 5 min unpunished period alternating with a 2 min punished period signalled by the light above the left lever. In the unpunished period reinforcement could be obtained by lever pressing according to a VI30 schedule of reinforcement. In the punished period responses produced a reward paired to a 0.5 s scrambled foot-shock according to a FR10 schedule of reinforcement. Rats underwent daily (Monday to Friday) training sessions, each consisting of six unpunished and six punished periods (total 42 min per session). Shock intensities were titrated individually by 0.05 mA until criterion was reached (mean = 0.765 mA, range = 0.55–1.05 mA, at the beginning of drug testing). Criterion performance was defined as less than 25 punished responses and at least 500 unpunished responses made by each individual animal per session. In the course of testing, shock intensities were adjusted when necessary.

2.4. Test procedure

Animals performing according to criterion on the previous training day were tested. The drugs were tested in the following order: chlordiazepoxide, alprazolam, flesinoxan, antagonism of flesinoxan by WAY-100635, WAY-100635, 8-OH-DPAT, antagonism of 8-OH-DPAT by WAY-100635, and quinpirole. For each drug the doses, including the vehicle control, were counterbalanced across subjects. Test sessions were identical to the training sessions, and were carried out on Wednesdays and Fridays for chlordiazepoxide and alprazolam. In the case of the 5-HT_{1A}

receptor compounds (8-OH-DPAT, flesinoxan, WAY-100635) and quinpirole, administration and testing occurred only once a week to prevent any desensitization to the drug (Groenink et al., 1997).

2.5. Data analysis

The number of punished and unpunished responses was recorded, and the results are expressed as mean number of lever presses ± S.E.M. Multivariate analysis of variance (MANOVA) of the statistical package for the social sciences (SPSS-pc) was used for statistical analysis. Each animal served as its own control and the punished and the unpunished responses were tested separately. We predicted a dose-dependent effect of chlordiazepoxide, alprazolam, 8-OH-DPAT and flesinoxan on the punished response, and tested the significance of the linear component of this effect by means of a polynomial analysis of variance with one within-subjects variable (drug dose). All other data were analyzed using the averaged test of the univariate analysis of variance with repeated measures on one factor (drug dose). When the Mauchly test for sphericity was significant, an adjustment was made on the numerator and the denominator for the degrees of freedom using the Greenhouse-Geisser epsilon. When the univariate test was significant, the significance of the individual doses were tested with a univariate *F*-test followed by a Bonferroni correction on the significance level. A significance level of 0.05 was considered significant throughout the statistical analysis.

2.6. Drugs

Chlordiazepoxide hydrochloride (OPG, Utrecht, Netherlands), flesinoxan (*R*(+)-*N*-[2[4-(2,3-dihydro-2-2-hydroxymethyl-1,4-benzodioxin-5-yl)-1-piperazinyl]ethyl]-4-fluorobenzoamide) (Solvay-Duphar, Weesp, Netherlands), WAY-100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl) cyclohexanecarboxamide trihydrochloride) (synthesized by Solvay-Duphar) and 8-OH-DPAT (8-hydroxy-2-(di-*n*-propyl-amino)tetralin HBr) (Research Biochemicals International, Natick, MA, USA) were dissolved in 0.9% saline. Alprazolam (Upjohn, Kalamazoo, MI, USA) and quinpirole hydrochloride (Research Biochemicals International) were suspended in gelatin mannitol (0.5% gelatin, 5% mannitol). All drugs were administered s.c. 30 min prior to testing, with an injection volume of 2 ml/kg.

3. Results

3.1. Benzodiazepines

Chlordiazepoxide elicited a dose-dependent linear increase in punished responding during the conflict period ($F(1,14) = 27.8$), with a maximum effect at 20.0 mg/kg (38.5 punished responses vs. 16.7 for controls) (Fig. 1, left). The univariate analysis indicated no significant effect of chlordiazepoxide on the unpunished response ($F(5,70) = 2.2$).

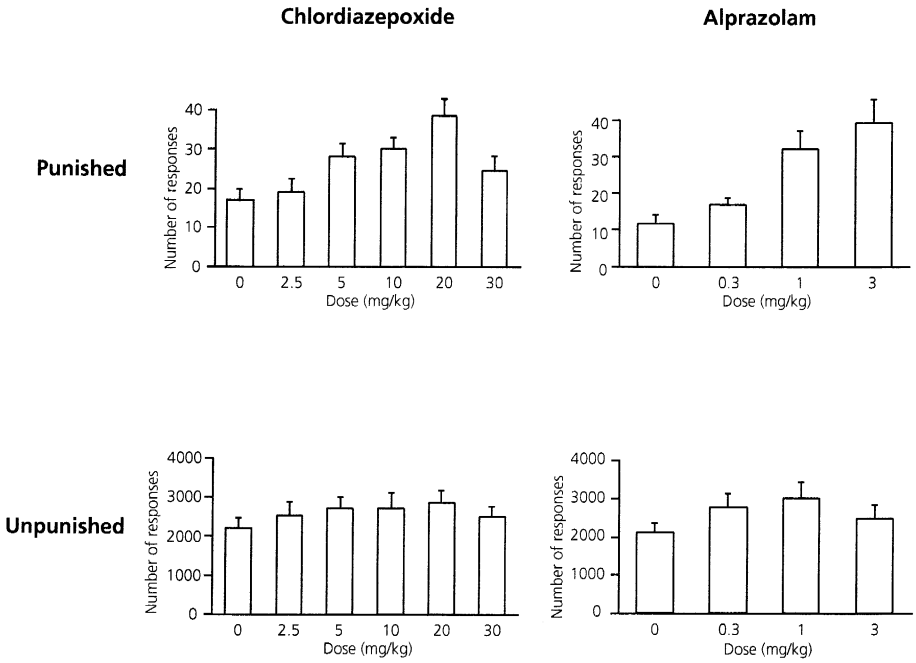

Fig. 1. Effect of chlordiazepoxide (left) and alprazolam (right) on punished and unpunished response. Each dose was tested in 13–15 rats and results are expressed as mean number of lever presses ± S.E.M. Please note that the *y*-axes for the benzodiazepines and the 5-HT_{1A} compounds (Figs. 2 and 3) are different. Chlordiazepoxide and alprazolam induced a significant dose-dependent increase in punished responding, and alprazolam significantly increased unpunished responding.

Alprazolam produced a dose-dependent linear increase in punished responding ($F(1,12) = 24.1$), with a maximum effect at 3 mg/kg (37.5 punished responses vs. 11.5 control values) (Fig. 1, right). Alprazolam had a significant effect on unpunished responding ($F(2,25) = 5.8$), and pairwise comparison revealed that the 0.3 mg/kg ($F(1,12) = 9.0$) and 1 mg/kg ($F(1,12) = 12.7$) doses significantly increased punished responding.

3.2. 5-HT_{1A} receptor ligands

The linear polynomial analysis indicated that 8-OH-DPAT had a significant effect on the punished response ($F(1,15) = 4.4$). 8-OH-DPAT increased punished responding and the strongest effect was seen at 0.5 mg/kg, where the number of responses increased from 8.2 (control) to 18.2 (Fig. 2, left). Univariate analysis indicated that 8-OH-DPAT had a significant effect on unpunished behaviour ($F(5,75) = 31.6$). The response was significantly reduced at 0.2 mg/kg ($F(1,15) = 22.5$), 0.3 mg/kg ($F(1,15 = 43.8$) and 0.5 mg/kg ($F(1,15) = 83.0$) 8-OH-DPAT, bringing control values of 2040.6 down to ultimately 226.8 lever presses.

Flesinoxan gave rise to a significant linear increase in punished responding ($F(1,15) = 7.6$). The number of responses increased from 9.25 (control) to 19.4 responses (Fig. 2, middle). The univariate analysis showed that flesinoxan had a marked depressant effect on unpunished responding ($F(3,43) = 39.0$). Specifically, unpunished responding was significantly reduced at the doses of 1.0 mg/kg ($F(1,15) = 18.7$), 3.0 mg/kg ($F(1,15) = 28.6$) and 10.0 mg/kg ($F(1,15) = 77.5$), bringing the control value of 2264.1 down to ultimately 196.9 lever presses.

WAY-100635 did not have a significant effect on punished responding ($F(5,70) = 0.8$) (Fig. 2, right). It did have a significant effect on unpunished responding ($F(5,70) = 36.3$). Pairwise comparison indicated that this was significant at the doses 1.0 mg/kg ($F(1,14) = 29.6$) and 3.0 mg/kg ($F(1,14) = 114.9$), and at the highest dose (3 mg/kg) response was reduced from 2263.0 (control) to 321.8 lever presses.

3.3. Antagonism studies

The 0.3 mg/kg dose of 8-OH-DPAT was selected for antagonism. Univariate analysis indicated that WAY-100635 had a significant effect on punished responding induced by 0.3 mg/kg 8-OH-DPAT ($F(2,34) = 5.3$). More specifically, pairwise comparison showed that 8-OH-DPAT alone significantly increased punished responding ($F(1,14) = 19.1$), and this effect could only be antagonized with the highest dose of WAY-100635 (1.0 mg/kg, $F(1,14) = 8.2$) (Fig. 3, left). WAY-100635 also had a significant effect on the unpunished responding induced by 0.3 mg/kg 8-OH-DPAT ($F(2,9) = 18.3$). More specifically, pairwise comparison indicated that 8-OH-DPAT alone decreased unpunished responding ($F(1,14) = 36.5$), and this could be antagonized with every dose WAY-100635 used, namely 0.1

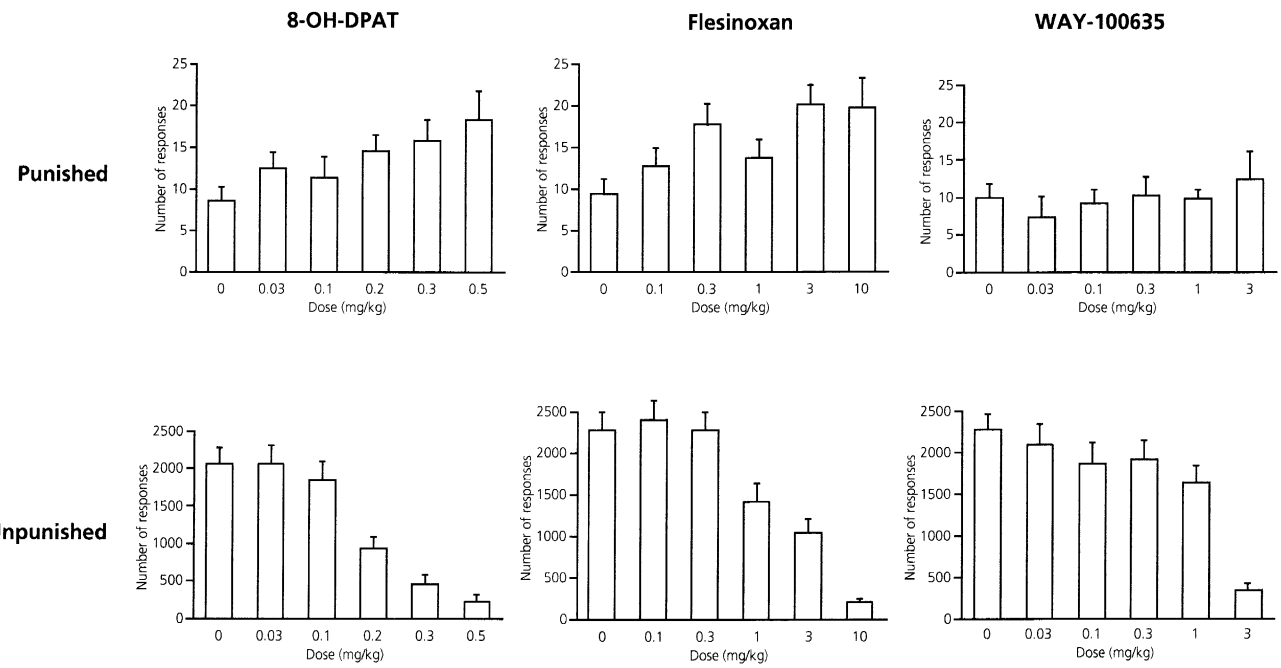

Fig. 2. Effect of 8-OH-DPAT (left), flesinoxan (middle) and WAY-100635 (right) on punished and unpunished response. Each dose was tested in 15–16 rats and the results are expressed as mean number of lever presses ± S.E.M. 8-OH-DPAT and flesinoxan induced a dose-dependent increase in punished responding. WAY-100635 had no effect on punished responding. 8-OH-DPAT, flesinoxan and WAY-100635 significantly decreased unpunished responding.

mg/kg ($F(1,14) = 21.5$), 0.3 mg/kg ($F(1,14) = 3.8$) or 1.0 mg/kg ($F(1,14) = 19.9$).

The 3.0 mg/kg dose of flesinoxan was selected for antagonism. Univariate analysis indicated that WAY-100635 had a significant effect on punished responding induced by 3.0 mg/kg flesinoxan ($F(3,38) = 5.6$). More specifically, pairwise comparison revealed that flesinoxan alone significantly increased punished responding ($F(1,14) = 17.8$), and this effect could only be antagonized with the highest dose of WAY-100635 (3.0 mg/kg, $F(1,14) = 10.4$) (Fig. 3, right). WAY-100635 also had a significant effect on the unpunished responding induced by 3.0 mg/kg flesinoxan ($F(3,39) = 27.4$). More specifically, pairwise comparison indicated that flesinoxan alone significantly decreased unpunished responding ($F(1,14) = 37.0$), and that this effect could be antagonized with intermediate doses of WAY-100635 used, namely 0.1 mg/kg ($F(1,14) = 44.8$), 0.3 mg/kg ($F(1,14) = 18.4$) and 1.0 mg/kg ($F(1,14) = 20.4$); but not with the highest dose (3.0 mg/kg).

3.4. Quinpirole

The effect of the dopamine D_3/D_2 receptor agonist quinpirole in a dose range of 0.025–0.10 mg/kg s.c. on the punished response was not significant ($F(3,42) = 1.7$). Quinpirole did have a significant effect on the unpunished response ($F(3,42) = 10.0$), decreasing responding at 0.025 mg/kg ($F(1,14) = 10.9$), 0.05 mg/kg ($F(1,14) = 29.4$) and 0.1 mg/kg ($F(1,14) = 12.00$) (data not shown).

4. Discussion

A substantial body of research using conflict procedures has shown the benzodiazepine chlordiazepoxide to consistently release punished responding while hardly affecting unpunished behaviour until high doses are administered (Pollard and Howard, 1990, for a review). In the present experiment both chlordiazepoxide and alprazolam produced a marked dose-dependent increase in punished responding. Our results are in accordance with previous investigations, and agree with the long-held notion that the conflict paradigm is effective and consistent in screening for anxiolytics acting at the benzodiazepine receptor (Carlton, 1983; Sanger, 1990).

The main purpose of the present experiment was to determine the putative anxiolytic activity of 5-HT_{1A} receptor agonists in a conflict paradigm, and to verify whether such an effect is mediated by the 5-HT_{1A} receptor. The 5-HT_{1A} receptor agonist 8-OH-DPAT elicited a significant increase in punished responding, extending the results previously reported by Schreiber and De Vry (1993). Furthermore, another 5-HT_{1A} receptor agonist, flesinoxan, exerted a significant dose-dependent increase in punished responding. The effects of 0.3 mg/kg 8-OH-DPAT and

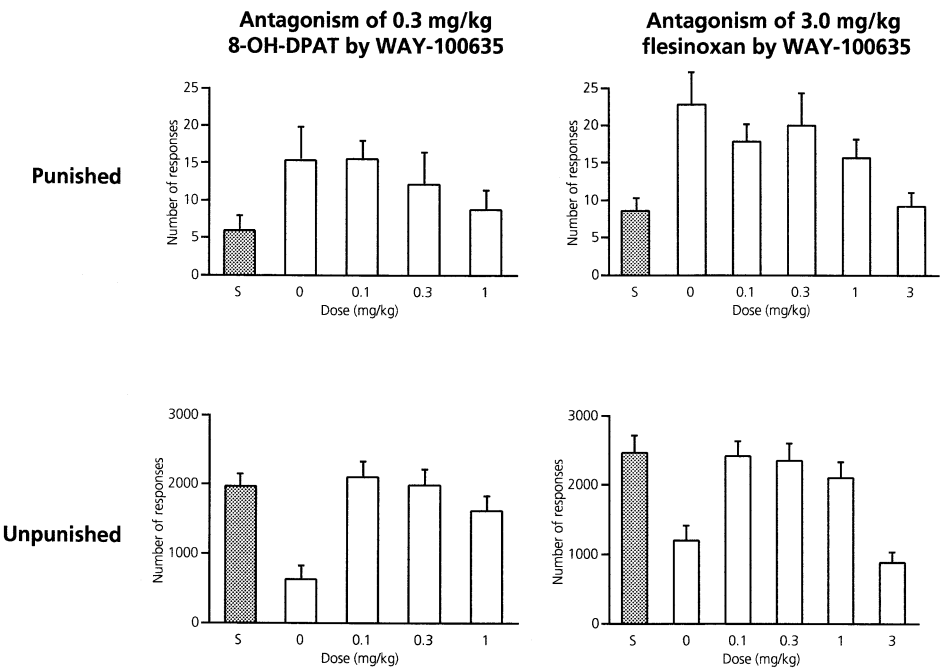

Fig. 3. Antagonism by WAY-100635 of the effect of 0.3 mg/kg 8-OH-DPAT (left) and 3.0 mg/kg flesinoxan (right), on punished and unpunished responses. Each dose was tested in 15–16 rats and the results are expressed as mean number of lever presses ± S.E.M. The doses WAY-100635 are read on the abscissa and the grey bar indicates the double-saline (0 mg/kg flesinoxan or 8-OH-DPAT + 0 mg/kg WAY-100635) condition. 8-OH-DPAT and flesinoxan increased punished responding and this could only be antagonized with the highest dose of WAY-100635 (1.0 mg/kg and 3.0 mg/kg respectively). 8-OH-DPAT and flesinoxan also reduced unpunished response and this could be antagonized with every dose of WAY-100635, except the 3.0 mg/kg dose.

3.0 mg/kg flesinoxan could be replicated in the antagonism studies (Fig. 3), supporting a role for both 8-OH-DPAT and flesinoxan as anxiolytic-like compounds. WAY-100635, a highly selective 5-HT_{1A} receptor antagonist, did not show any intrinsic anxiolytic activity.

The effects of both 8-OH-DPAT and flesinoxan on unpunished behaviour were quite pronounced, reducing unpunished responding significantly at the higher doses. The present results are in accordance with previous reports of significant inhibition of the unpunished response in conflict models after administration of the 5-HT_{1A} receptor agonists 8-OH-DPAT, ipsapirone and gepirone (Cervo and Samanin, 1995; Sanger, 1990; Young et al., 1987). WAY-100635, on itself, had no effect on the unpunished response with the exception of the highest dose (3.0 mg/kg) which dramatically reduced responding.

The antagonism experiments showed that all doses of WAY-100635 were able to antagonize the response-suppressing effect of 8-OH-DPAT on unpunished responding and bring it back to values comparable to control levels. This is in agreement with a previously reported antagonism by WAY-100635 of the 8-OH-DPAT-induced 5-HT behavioural syndrome (Fletcher et al., 1996). Similarly, when administered with flesinoxan, all doses of WAY-100635 fully antagonized the response-suppressing effect in unpunished periods. This complete antagonism confirms that 8-OH-DPAT and flesinoxan's inhibitory effect on the unpunished behaviour is due to their agonistic action at 5-HT_{1A} receptors.

On the other hand, the releasing effect of 8-OH-DPAT on punished responding was unaltered by the two intermediate doses of WAY-100635, and significant antagonism occurred only at the highest dose (1.0 mg/kg). Similarly, no intermediate dose of WAY-100635 reduced the anti-conflict effect of flesinoxan in the punished period, and again only the highest dose (3.0 mg/kg) reduced punished responding to values comparable to control. It is evident from Fig. 3 that the antagonism of flesinoxan by 3.0 mg/kg WAY-100635 on the punished response is accompanied by a simultaneous dramatic inhibition of the unpunished response. This concordance alone precludes any conclusion about the involvement of the 5-HT_{1A} receptor in the anxiolytic effect of 8-OH-DPAT and flesinoxan.

It does not seem likely that the selected dose-range is too narrow, as previous research obtained antagonism of 8-OH-DPAT with lower doses of WAY-100635 than the ones used here. Fletcher et al. (1996) showed that hypothermia induced by 0.25 mg/kg s.c. 8-OH-DPAT (a dose comparable to ours) could be antagonized by WAY-100635 in a dose range of 0.01–0.1 mg/kg. In addition 0.1 mg/kg 8-OH-DPAT induced hyperphagia, increased ACTH and reduced the number of trials completed in a delayed matching to position task, and all these effects could be antagonized by WAY-100635 with doses as low as 0.01–0.1 mg/kg. Finally, the discriminative stimulus of a relatively high dose of 8-OH-DPAT (0.4 mg/kg, i.p.), could be antagonized with a low dose of WAY-100635 (ED_{50} = 0.017 mg/kg, i.p., range: 0.012–0.024 mg/kg, i.p., Piesla and Marquis, 1994). Taken together these reports do not support the need for a high dosage of WAY-100635 for antagonism.

It has been argued that anxiolytic effects of 5-HT_{1A} receptor agonists are mediated by somatodendritic 5-HT_{1A} receptors in the raphe nuclei (Jolas et al., 1995; Cervo and Samanin, 1995; De Vry, 1995). Given the important receptor reserve in the raphe (Meller et al., 1990), a high dose of the antagonist may be needed to block the effects of a 5-HT_{1A} receptor agonist. However, not only post-synaptic-mediated effects, such as 8-OH-DPAT-induced hypothermia (Bill et al., 1991) and the discriminative stimulus of 8-OH-DPAT (Kalkman, 1990) could be antagonized by low doses of WAY-100635 (Fletcher et al., 1996; Piesla and Marquis, 1994), but also effects mediated by pre-synaptic receptors, such as 8-OH-DPAT-induced hyperphagia (Bendotti and Samanin, 1986; Hutson et al., 1986) were antagonized by low doses of WAY-100635 (Fletcher et al., 1996). Therefore, it remains questionable whether the antagonism of 8-OH-DPAT and flesinoxan by WAY-100635 reflects true pre-synaptic 5-HT_{1A} receptor blocking effects.

Using a variant of the Geller-Seifter model, Charrier et al. (1994) found a similar discrepancy between the complete antagonism of the unpunished response and the inability to antagonize the anxiolytic-like effect of a 5-HT_{1A} receptor agonist. In this conflict model, where the punished period was signalled by the withdrawal of a discriminative ('safety') stimulus, the response releasing effect of a low (0.125 mg/kg, s.c.), but not of a higher (0.25 mg/kg, s.c.), dose of buspirone could be antagonized by the 5-HT_{1A} receptor antagonist WAY-100135 (5.0 mg/kg, s.c.). In the fear-potentiated startle paradigm, buspirone (Davis et al., 1988) and flesinoxan (Joordens et al., 1996) have anxiolytic-like properties. However, this effect of flesinoxan could not be antagonized by WAY-100635 (Joordens, manuscript in preparation) and Davis et al. found no evidence supporting a role for the 5-HT_{1A} receptor in the mediation of buspirone's anxiolytic effect. Finally, Groenink et al. (1996) reported that WAY-100635 did not antagonize the effects of flesinoxan on plasma prolactin levels. Taken together, these reports question the involvement of the 5-HT_{1A} receptor in some of the anxiolytic or stress effects of buspirone and flesinoxan.

Possibly other receptors may be involved in the mediation of the effect of 8-OH-DPAT and flesinoxan on the punished response, i.e. their potential anxiolytic effect. In the present experiment the dopamine D_2/D_3 receptor agonist quinpirole did not elicit a consistent increase in punished responding, which does not support a role for the dopamine D_3 or D_2 receptor subtypes in mediating the effect of flesinoxan. The affinity of flesinoxan for various other receptors is even weaker, and there have been no reports indicating that the anxiolytic activity of flesinoxan

may involve receptors other than 5-HT_{1A} (Schipper et al., 1991).

In conclusion, the present experiment provides further evidence supporting 8-OH-DPAT and flesinoxan as anxiolytic drugs. However, the observed pattern of antagonism of their effects with WAY-100635 suggests that the activation of the 5-HT_{1A} receptor subtype cannot entirely account for their anxiolytic activity.

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