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European Journal of Pharmacology 334 (1997) 25–29

ejp

Short communication

Repeated DOI and SR 46349B treatments do not affect elevated plus-maze anxiety despite opposite effects on cortical 5-HT_{2A} receptors

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Received 12 May 1997; revised 22 July 1997; accepted 25 July 1997

Abstract

We report the consequences of a 4-day treatment (b.i.d) with the 5-$HT_{2A,2B,2C}$ receptor agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI, 1.5 mg/kg) or the selective 5-HT_{2A} receptor antagonist *trans*-4-[(3*Z*)3-(2-dimethylaminoethyl)oxyimino-3-(2-fluorophenyl)propen-1-yl]phenol hemifumarate (SR 46349B, 7.5 mg/kg) on (i) anxiety-related behaviour in an elevated plus-maze, and (ii) specific [^{3}H]ketanserin binding at central 5-HT_{2A} receptors, in Roman rats. Neither DOI nor SR 46349B pretreatment affected the behaviour in the open arms of the elevated plus-maze; however, DOI pretreatment promoted discrete changes in the closed arm entries. The B_{max} value of [^{3}H]ketanserin binding at cortical 5-HT_{2A} receptors was decreased by repeated DOI pretreatment. Conversely, B_{max}, but also K_D, values were increased by SR 46349B pretreatment. Thus, changes at central 5-HT_{2A} receptors may occur without there being changes in anxiety-related behaviour in the elevated plus-maze. © 1997 Elsevier Science B.V.

Keywords: DOI (1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane); SR 46349B; 5-HT_{2A} receptors; Anxiety; Elevated plus-maze; [^{3}H]ketanserin binding; RHA and RLA (Roman high- and low-avoidance) rats

1. Introduction

The lack of availability of selective ligands for numerous 5-HT receptor types has hampered the recognition of their respective roles, if any, in the aetiology of anxiety. Among these receptors, the 5-HT_{2A} receptor is of interest as early clinical data have suggested that its blockade could lead to anxiolysis (Ceulemans et al., 1985). However, this suggestion emerged from studies involving ritanserin, a 5-$HT_{2A,2B,2C}$ receptor antagonist (Baxter et al., 1995). In keeping with the acute anxiolytic effect of 5-$HT_{2B,2C}$ receptor blockade in animals (Kennett et al., 1996), the hypothesis that 5-HT_{2A} receptors do not mediate the clinical effects of ritanserin is noteworthy. This hypothesis is reinforced when one considers the results of animal studies aimed at analysing the role of 5-HT_{2A} receptors in anxiety. Actually, acute administration of the widely used 5-HT_{2A} receptor agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) has been reported to exert anxiolytic, anxiogenic, or no effects at all (see Griebel, 1995). However, the interpretation of DOI-related studies is complicated by the finding that DOI also binds to 5-$HT_{2B,2C}$ receptors (Baxter et al., 1995), the acute stimulation of which leads to anxiety (Kennett et al., 1989). Moreover, acute administration of the preferential 5-HT_{2A} receptor antagonists ketanserin and RP 62203 (Kennett, 1993) decreases or does not affect anxiety-related behaviours (see Griebel, 1995). This uncertainty concerning the acute role of 5-HT_{2A} receptors on anxiety extends to repeated/chronic manipulations of this receptor. The behavioural effects of repeated treatments with selective 5-HT_{2A} receptor antagonists in animal models of anxiety have not yet been reported. Acute pretreatment (48 h beforehand) with the 5-$HT_{2A,2B,2C}$ receptor antagonist mianserin or repeated pretreatment (last pretreatment 24 h beforehand) with the 5-$HT_{2A,2B,2C}$ receptor antagonist ritanserin has been shown to elicit anxiolysis in the elevated plus-maze (Benjamin et al., 1992; Wright et al., 1992), an effect associated with 5-HT_{2A} receptor down-regulation (Leysen et al., 1986; Benjamin et al., 1992). However, knock-out mice lacking 5-HT_{2C} receptors display decreased anxiety in the elevated zero-maze (Tecott, 1996),

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thus questioning again the role of 5-HT_{2A} receptors in the behavioural effects of ritanserin and mianserin.

The aim of this work was thus to investigate the effects of repeated treatment with the selective 5-HT_{2A} receptor antagonist *trans*-4-[(3*Z*)3-(2-dimethylamino-ethyl)oxyimino-3-(2-fluorophenyl)propen-1-yl]phenol hemifumarate (SR 46349B; Rinaldi-Carmona et al., 1992 Rinaldi-Carmona et al., 1993a,b) in the elevated plus-maze test of anxiety. In addition, because the effects of repeated stimulation of 5-$HT_{2A,2B,2C}$ receptors on anxiety are not yet known, the effects of SR 46349B were compared with those of DOI. To verify that these treatments were endowed with opposite effects on 5-HT_{2A} receptors (McKenna et al., 1989; Chaouloff et al., 1993; Rinaldi-Carmona et al., 1993a,b), [^{3}H]ketanserin binding to cortical 5-HT_{2A} receptors was also studied. Lastly, to investigate whether the effects of DOI and SR 46349B on elevated plus-maze behaviours (if any) and 5-HT_{2A} receptors depend on the baseline levels of these variables, we performed our experiments with Roman high- (RHA) and low-avoidance (RLA) rats. Thus, using Roman rats (Bordeaux sublines), we recently reported that RHA and RLA rats displayed high and low cortical [^{3}H]ketanserin binding and high and low anxiety scores in the elevated plus-maze, respectively (Kulikov et al., 1995). However, because of the high number of animals required for the present study, we used RHA and RLA rats of the original subline (RHA/Verh and RLA/Verh).

2. Materials and methods

2.1. Animals and housing conditions

The animals used in the present study have been bred for one generation from rats of the RHA/Verh and RLA/Verh lines, kindly provided by Dr P. Driscoll (Swiss Federal Institute of Technology, Zürich, Switzerland). Animals were weaned at 3 weeks and randomly housed, four per cage, by line and sex. All cages were placed in a room with constant temperature (22 ± 1°C) and a 12 h:12 h cycle of illumination (lights on: 07.00 h).

2.2. Experimental procedure

When 10–12 weeks old, 36 males of each line were weighed and injected i.p. either with vehicle (see below), with DOI (1.5 mg/kg), or with SR 46349B (7.5 mg/kg) for 5 days ($n = 12$/pretreatment group/line). The doses of the compounds were chosen according to previous studies (McKenna et al., 1989; Chaouloff et al., 1993; Rinaldi-Carmona et al., 1993a,b). DOI (R.B.I., BioBlock, Illkirch, France) and SR 46349B (Sanofi Recherche, Montpellier, France) were dissolved in few drops of ethanol and diluted as required in distilled water. Each treatment was administered in the early afternoon of the first day, in the early morning and afternoon of the second, third, and fourth days, and in the early morning of the fifth day. Each rat thus received one series of 8 treatments. On the sixth day, the rats were submitted to an elevated plus-maze test in the morning (24 h after the last drug injection) and were killed by decapitation in mid-afternoon for the dissection of frontal cortices.

2.3. Elevated plus-maze tests

The elevated plus-maze was made of Perspex® with 2 opposite open arms (45×10 cm), and 2 opposite closed arms of the same size with walls 50 cm high. The arms were connected by a central square (10×10 cm). In addition, because the floor surface of the maze was smooth, rubber ridges bordering the open arms (0.5 cm) were added to provide additional grip for the animals. The whole apparatus was elevated 66 cm above a white floor and exposed to dim illumination (70 lux). The testing procedure was similar to that previously described (Kulikov et al., 1995; Ramos et al., 1997). Thus, vehicle-, DOI-, and SR 46349B-treated RHA and RLA rats were randomly placed in the central square of the maze, facing an open arm. The number of entries onto and time spent on each arm were then video-monitored for 5 min (an arm entry being defined as all four feet on the arm). At the end of each test, the rat was returned to its home cage. Note that in our hands, such a procedure allows the detection of the anxiolytic and anxiogenic effects of diazepam and pentylenetetrazole, respectively (Ramos et al., 1997).

2.4. [^{3}H]ketanserin binding assays

As stated above, vehicle-, DOI-, and SR 46349B-pretreated rats from each line ($n = 12$/drug treatment) were killed 6 h after completion of the elevated plus-maze tests (i.e. 30 h after the last drug injection). Frontal cortices were rapidly dissected out and stored at −80°C until analysis. Frontal cortices from 2 animals (same treatment, same line) were pooled, and homogenised in 40 vol of ice-cold Tris–HCl buffer (0.05 M, pH 7.7). Homogenates were then centrifuged at $15\,000 \times g$ for 15 min (4°C). The resulting pellets were resuspended in 40 vol of buffer and centrifuged, as mentioned above. The final pellets were stored at −80°C until radioligand binding experiments (Kulikov et al., 1995). The pellets were suspended in 40 vol of ice-cold Tris–HCl buffer (0.05 M, pH 7.7), and the suspension (0.47 ml) was transferred to glass tubes. Radioligand binding assays were performed by means of a 15-min incubation at 35°C in the presence of 0.25–4 nM [^{3}H]ketanserin (63.7 Ci/mmol; Dupont/NEN, Les Ulis, France). Non-specific binding was assessed by adding 10 μM methysergide (R.B.I., BioBlock, Illkirch, France) to the test tubes. All assays were stopped by addition of 4 ml of cold buffer followed by rapid filtration through Whatman GF/B glass fibre filters. The filters were then washed

twice with 4 ml of the buffer, and radioactivity was counted by liquid scintillation. Protein concentrations were measured using bovine serum albumin as a standard (Bradford, 1976). All samples were assayed in duplicate, and the data analysed by means of Scatchard plots.

2.5. Statistics

The values are shown as means ± S.E.M. Percentages of entries onto and time spent on open arms of the elevated plus-maze were analysed by a Kruskal–Wallis analysis of variance for non-parametric data. The other data (including closed and total arm entries in the elevated plus-maze) were compared by means of 2-way analyses of variance (with the strain and the pretreatment as main factors) followed, if significant ($P < 0.05$), by Tukey's comparison tests. For radioligand binding data, these analyses of variance followed linear regression analyses (least-squares method) for determination of B_{max} and K_D values.

3. Results

3.1. Effects of DOI and SR 46349B pretreatments on body weight in RHA and RLA rats

Analyses of the respective effects of the 4-day pretreatments on body weight revealed no strain effect, but a significant decrease in DOI-pretreated rats (0.6 ± 0.3% and 0.75 ± 0.81% decreases in RHA and RLA rats, respectively) and, to a lesser extent, in SR 46349B-pretreated rats (1.47 ± 0.39% and 1.47 ± 0.47% increases in RHA and RLA rats), compared with vehicle-pretreated rats (4.18 ± 0.53% and 3.44 ± 0.3% increases in RHA and RLA rats, respectively; $P < 0.01$ for the differences with DOI and SR 46349B in each strain). Actually, a significant decrease in body weight occurred after the first injection of SR 46349B, whereas DOI decreased body weight until the fourth injection (data not shown).

3.2. Effects of DOI and SR 46349B pretreatments on elevated plus-maze behaviours in RHA and RLA rats

Neither the percent number of open arm visits nor the percent time spent on open arms varied significantly among the six rat groups (Fig. 1). However, when rats were grouped by strain (RHA or RLA), the percent time spent on open arms (but not the percent number of visits thereon) was higher in RLAs (22.4 ± 4.4) than in RHAs (11.3 ± 1.5, $P = 0.028$). The influence of the strain factor extended to the number of closed arm entries ($F = 12.8$, $P = 0.001$), and to the number of total arm entries ($F = 7.3$, $P = 0.009$), with RLA rats displaying fewer closed and total arm entries than RHA rats (Fig. 1). Lastly, there was a significant strain × pretreatment interaction on the number of closed arm entries ($F = 5.43$, $P = 0.007$), as illustrated by the inhibitory effect of DOI on this variable in RLA rats (Fig. 1).

3.3. Effects of DOI and SR 46349B pretreatments on [^{3}H]ketanserin binding in frontal cortices of RHA and RLA rats

As shown in Fig. 2, the pretreatment, but not the strain, affected [^{3}H]ketanserin binding at frontal cortex 5-HT$_{2A}$ receptors ($F = 28.1$, $P < 0.0001$ and $F = 4.58$, $P = 0.018$

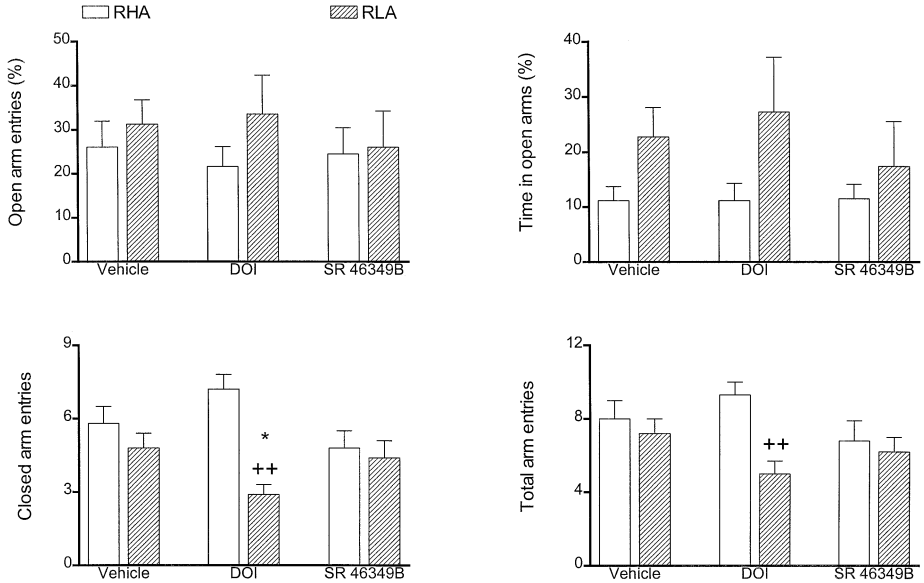

Fig. 1. Effects of repeated DOI and SR 46349B treatments in RHA and RLA rats exposed to a 5-min elevated plus-maze test. Values are the means ± S.E.M. for 11–12 rats. * $P < 0.05$ for the effect of DOI in RLA rats; ++ $P < 0.01$ for the strain effect in DOI-pretreated rats. See text for strain-related differences.

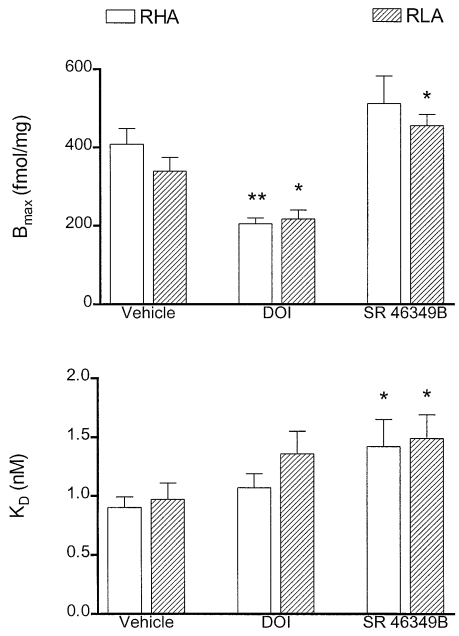

Fig. 2. Effects of repeated DOI and SR 46349B treatments on [^{3}H]ketanserin-specific binding in the frontal cortices of RHA and RLA rats. Values are the means ± S.E.M. for 6 assays. * $P < 0.05$ and ** $P < 0.01$ for the difference with vehicle.

for B_{max} and K_D values, respectively). Actually, DOI pretreatment decreased B_{max} values in both strains, whereas the increase in B_{max} elicited by SR 46349B (which was associated with an increase in K_D values) was significant in RLA rats only (Fig. 2).

4. Discussion

We have previously reported that RLA rats (i) entered more often, and spent more time, on the open arms of an elevated plus-maze, and (ii) displayed decreased [^{3}H]ketanserin binding at cortical 5-HT$_{2A}$ receptors, compared with RHA rats (Kulikov et al., 1995). In this study, the percent time spent on the open arms of the plus-maze was the sole variable that displayed a significant strain-dependent profile. This discrepancy may be related to the rats' origin, as the present study used rats bred for one generation from the original Verh sublines instead of rats bred for years in our laboratory; however, factors of environmental origin may also partly/totally underlie such a discrepancy. Thus, subchronic stress (single housing) was found to reduce and prevent the aforementioned anxiety- and 5-HT$_{2A}$ receptor-related differences between Roman lines, suggesting that RLA rats may be more sensitive to stress than their RHA counterparts (Kulikov et al., 1995). If true, repeated i.p. vehicle injection, i.e. a paradigm that may promote anxiety in the elevated plus-maze (Wright et al., 1992), could underlie the discrepancy between our past and present studies.

On repeated treatment, SR 46349 up-regulates 5-HT$_{2A}$ receptors, as evidenced by radioligand binding and second messenger studies (Rinaldi-Carmona et al., 1993a,b). This was confirmed in the present study where repeated pretreatment with SR 46349B increased the number of cortical 5-HT$_{2A}$ receptors (in a strain-independent manner). However, this pretreatment increased the K_D values of cortical [^{3}H]ketanserin-specific binding by 53–57% in Roman rats. It is noteworthy that when administered according to a schedule similar to that presented herein (except for the route of administration: i.p. for p.o.), SR 46349B (10 mg/kg) caused a non-significant 53% increase in the K_D values of cortical [^{3}H]ketanserin binding (compared to vehicle-treated controls) in mice (Rinaldi-Carmona et al., 1993a) but not in rats (Rinaldi-Carmona et al., 1993b).

Repeated administration of SR 46349B affected neither the number of open arm entries nor the time spent on the open arms of the elevated plus-maze. This result contrasts with that obtained in a study where repeated administration of ritanserin was found, 24 h after the last treatment, to promote anxiolysis in this test (Wright et al., 1992). The comparison of studies is rendered difficult due to protocol differences; however, the finding that repeated SR 46349B administration on the one hand, and repeated ritanserin administration on the other hand, exerted opposite effects on cortical 5-HT$_{2A}$ receptors is noteworthy. Hence, this could suggest that 5-HT$_{2A}$ receptor down-, but not up-regulation is a prerequisite for anxiolysis. Against this hypothesis is the observation that repeated DOI administration did not promote anxiolysis in the elevated plus-maze although it markedly down-regulated 5-HT$_{2A}$ receptors (thus confirming the results of previous studies: McKenna et al., 1989; Chaouloff et al., 1993). Although the behavioural impact of DOI-induced 5-HT$_{2B,2C}$ receptor down-regulation (if any) is unknown, our study strongly suggests that the anxiolytic effect of repeated ritanserin treatment is mediated by 5-HT$_{2B,2C}$ receptors. This suggestion is reinforced by the observation that the 5-HT$_{2A}$ receptor antagonist SR 46349B has only moderate affinity for 5-HT$_{2C}$ receptors and weak affinity for the 5-HT$_{2B}$ receptor (as assessed through rat stomach fundus preparations) (Rinaldi-Carmona et al., 1992).

A differential effect of DOI on the number of closed arm entries could be observed in RLA and RHA rats exposed to the elevated plus-maze. A genetic variability in the number of closed arms entered following acute DOI administration has already been reported (in C57/Bl6 and DBA mice: Onaivi et al., 1995). Because this behaviour reflects locomotor activity (Ramos et al., 1997), it suggests that 5-HT$_{2A}$, 5-HT$_{2B}$ and/or 5-HT$_{2C}$ receptor down-regulation affects locomotor activity. However, repeated stimulation of 5-HT$_{2B,2C}$ receptors does not affect basal activity (Freo et al., 1992), hence suggesting that 5-HT$_{2A}$ receptor down-regulation may promote changes in locomotor activity.

In conclusion, our results show that repeated blockade

of 5-HT_{2A} receptors does not have any impact on rats' behaviour in the elevated plus-maze of anxiety although it promotes changes at the receptor level. Because different models of anxiety capture different dimensions of emotivity (see Ramos et al., 1997), it remains to be investigated whether our observation extends to other models of anxiety, including those of conditioned anxiety.

Acknowledgements

We thank Dr. P. Driscoll (Zürich, Switzerland) and Sanofi Recherche (Montpellier, France) for the kind gift of Roman breeders and SR 46349B, respectively.

References

Baxter, G., Kennett, G., Blaney, F., Blackburn, T., 1995. 5-HT_2 Receptor subtypes: a family re-united?. Trends Pharmacol. Sci. 16, 105–110.

Benjamin, D., Saiff, E.I., Nevins, T., Lal, H., 1992. Mianserin-induced 5-HT_2 receptor down-regulation results in anxiolytic effects in the elevated plus-maze. Drug Dev. Res. 26, 287–297.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein–dye binding. Analyt. Biochem. 72, 248–254.

Ceulemans, D.L.S., Hoppenbrouwers, M.L.J.A., Gelders, Y.G., Reyntjens, A.J.M., 1985. The influence of ritanserin, a serotonin antagonist, in anxiety disorders: a double-blind placebo controlled study versus lorazepam. Pharmacopsychiatry 18, 303–305.

Chaouloff, F., Baudrie, V., Coupry, I., 1993. Behavioural and biochemical evidence that glucocorticoids are not involved in DOI-elicited 5-HT_2 receptor down-regulation. Eur. J. Pharmacol. 249, 117–120.

Freo, U., Halloway, H.W., Greig, N.H., Soncrant, T.H., 1992. Chronic treatment with *meta*-chlorophenylpiperazine (*m*-CPP) alters behavioral and cerebral metabolic responses to the serotonin agonists *m*-CPP and quipazine but not 8-hydroxy-2(di-*N*-propylamino)tetralin. Psychopharmacology 107, 30–38.

Griebel, G., 1995. 5-Hydroxytryptamine-interacting drugs in animal models of anxiety disorders: more than 30 years of research. Pharmac. Ther. 65, 319–395.

Kennett, G.A., 1993. 5-HT_{1C} Receptors and their therapeutic relevance. Curr. Opini. Invest. Drugs 2, 317–362.

Kennett, G.A., Whitton, P., Shah, K., Curzon, G., 1989. Anxiogenic-like effects of *m*CPP and TFMPP in animal models are opposed by 5-HT_{1C} receptor antagonists. Eur. J. Pharmacol. 164, 445–454.

Kennett, G.A., Wood, M.D., Bright, F., Cilia, J., Piper, D.C., Gager, T., Thomas, D., Baxter, G.S., Forbes, I.T., Ham, P., Blackburn, T.P., 1996. In vitro and in vivo profile of SB 206553, a potent 5-HT_{2C} /5-HT_{2B} receptor antagonist with anxiolytic-like properties. Br. J. Pharmacol. 117, 427–434.

Kulikov, A., Castanon, N., Mormède, P., Chaouloff, F., 1995. Cerebral tryptophan hydroxylase activity, and 5-HT_{1A} receptor, 5-HT_{2A} receptor, and 5-HT transporter binding in grouped and isolated Roman RHA and RLA rats: relationships with behaviours in two models of anxiety. Psychopharmacology 121, 385–395.

Leysen, J.E., Van Gompel, P., Gommeren, W., Woestenborghs, R., Janssen, P.A.J., 1986. Down-regulation of serotonin S_2-receptor sites in rat brain by chronic treatment with the serotonin-S_2 antagonists: ritanserin and setoperone. Psychopharmacology 88, 434–444.

McKenna, D.J., Nazarali, A.J., Himeno, A., Saavedra, J.M., 1989. Chronic treatment with (±)DOI, a psychotomimetic 5-HT_2 agonist, downregulates 5-HT_2 receptors in rat brain. Neuropsychopharmacology 2, 81–87.

Onaivi, E.S., Bishop-Robinson, C., Darmani, N.A., Sanders-Bush, E., 1995. Behavioral effects of (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane, (DOI) in the elevated plus-maze test. Life Sci. 57, 2455–2466.

Ramos, A., Berton, O., Mormède, P., Chaouloff, F., 1997. A multiple-test study of anxiety-related behaviours in six inbred rat strains. Behav. Brain Res. 85, 57–69.

Rinaldi-Carmona, M., Congy, C., Santucci, V., Simiand, J., Gautret, B., Neliat, G., Labeeuw, B., Le Fur, G., Soubrié, P., Brelière, J.C., 1992. Biochemical and pharmacological properties of SR 46349B, a new potent and selective 5-hydroxytryptamine$_2$ receptor antagonist. J. Pharmacol. Exp. Ther. 262, 759–768.

Rinaldi-Carmona, M., Congy, C., Simiand, J., Oury-Donat, F., Soubrié, P., Brelière, J.C., Le Fur, G., 1993a. Repeated administration of SR 46349B, a selective 5- hydroxytryptamine$_2$ antagonist, up-regulates 5-hydroxytryptamine$_2$ receptors in mouse brain. Mol. Pharmacol. 43, 84–89.

Rinaldi-Carmona, M., Bouaboula, M., Congy, C., Oury-Donat, F., Simiand, J., Shire, D., Casellas, P., Soubrié, P., Brelière, J.C., Le Fur, G., 1993b. Up-regulation of 5-HT_2 receptors in the rat brain by repeated administration of SR 46349B, a selective 5-HT_2 receptor antagonist. Eur. J. Pharmacol. 246, 73–80.

Tecott, L., 1996. Behavioral correlates of 5-HT_{2C} receptor gene inactivation. J. Psychopharmacol. 10 (Suppl. 3), abst. 223.

Wright, I.K., Heaton, M., Upton, N., Marsden, C.A., 1992. Comparison of acute and chronic treatment of various serotonergic agents with those of diazepam and idazoxan in the rat elevated X-maze. Psychopharmacology 107, 405–414.