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Repeated Administration of SR 46349B, a Selective 5-Hydroxytryptamine$_2$ Antagonist, Up-regulates 5-Hydroxytryptamine$_2$ Receptors in Mouse Brain

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SUMMARY

Adaptive changes in 5-hydroxytryptamine (5-HT)$_2$ receptors were investigated in mice after repeated administration of SR 46349B, a potent, selective, and competitive 5-HT$_2$ receptor antagonist (K_i = 0.72 ± 0.05 nM). Repeated administration (twice per day for 3 days and once on the morning of the fourth day) of SR 46349B (5 or 10 mg/kg, orally) caused 24 hr later a marked increase in 5-HT$_2$ receptor number (+41% and +75%, respectively), measured *ex vivo* in brain cortical membranes with [^{3}H] ketanserin, without affecting its affinity constant. Further, administration of the 5-HT$_2$ agonist (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane produced, in SR 46349B (10 mg/kg, orally)-treated mice, a significant stimulation of the 5-HT$_2$ receptor-linked phosphoinositide turnover *in vivo* in the brain. In addition, subacute administration of SR 46349B (5 or 10 mg/kg, orally) caused a significant increase of the head-twitch response to L-5-hydroxytryptophan. This enhanced response was blocked by an acute administration of ritanserin (1 mg/kg). These results show that repeated administration of SR 46349B produced a parallel enhancement in 5-HT$_2$ receptor number, in 5-HT$_2$ receptor-linked signal transduction, and in 5-HT$_2$ receptor-mediated behavioral responses in mice. These findings suggest for the first time that an up-regulation of 5-HT$_2$ receptors can occur after repeated treatment with a selective 5-HT$_2$ antagonist.

According to classical monoamine receptor adaptation theory, receptor down-regulation is known to occur after repeated administration of agonists, whereas chronic antagonist treatment causes receptor up-regulation. To date there is some evidence for the concept that the 5-HT$_2$ receptor exhibits unexpected regulation *in vivo*. It has been widely reported by others that the density of 5-HT$_2$ receptors in rodent brain is down-regulated in response to chronic treatment with antidepressant or neuroleptic drugs (1-3), with 5-HT$_2$ agonists, including (±)-DOI, 1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane, and 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (4-6), and paradoxically with 5-HT$_2$ antagonists like ketanserin, ritanserin, mianserin, cyproheptadine, and pizotifen (7-9). The chronic administration of these compounds produced a significant decrease in rat cortical 5-HT$_2$ receptor number without changing the affinity constant, suggesting that this decrease is probably not due to direct competition between drug and radioligand (10).

Recently, SR 46349B, a propenone oxime ether derivative (Fig. 1), was found to be a potent and highly selective 5-HT$_2$ receptor antagonist (11). In *in vitro* binding studies SR 46349B possessed a high affinity for brain 5-HT$_2$ receptors. This interaction was specific, inasmuch as this compound was devoid of affinity for a panel of receptors including α_1- and α_2-adrenergic, D_1 and D_2 dopaminergic, H_1 histaminergic, and 5-HT$_3$ and 5-HT$_1$ serotonergic subclasses of receptor. Furthermore, SR 46349B also had no detectable affinity for the neurotransmitter (5-HT, dopamine, or norepinephrine) uptake sites. In addition, it potently and selectively antagonized 5-HT-evoked contractile responses known to involve 5-HT$_2$ receptor activation in isolated tissues (rabbit thoracic aorta, rat jugular vein, rat caudal artery, and guinea pig trachea).

In order to further explore 5-HT$_2$ receptor regulation *in vivo*, we investigated the effects of chronic treatments with SR 46349B on 5-HT$_2$ receptor binding properties, 5-HT$_2$ receptor-mediated behavior, and the 5-HT$_2$ receptor-linked second messenger system, in mice.

Experimental Procedures

Materials. PMSF, bovine serum albumin, and DTT were purchased from Boehringer (Mannheim, Germany). L5-HTP was purchased from Sigma Chemical Co. (St. Louis, MO). Polyethylenimine was purchased from Serva (St. Germain-en-Laye, France). Biofluor liquid scintillant and [^{3}H]ketanserin (60 Ci/mmol) were purchased from New England Nuclear Corporation (Paris, France). Ketanserin tartrate, (±)-DOI,

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; DMSO, dimethylsulfoxide; (±)-DOI, (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; DTT, dithiothreitol; L-5-HTP, 5-hydroxytryptophan; PMSF, phenylmethylsulfonyl fluoride.

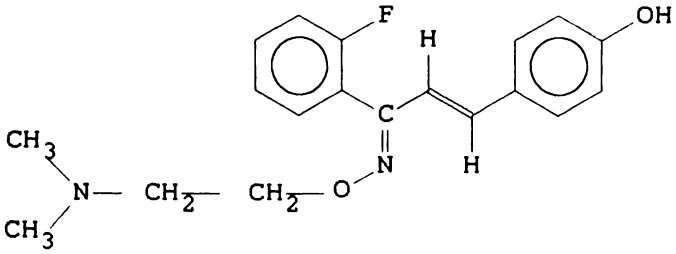

Fig. 1. Structure of SR 46349B.

and ritanserin were purchased from RBI (Illkirch, France). *myo*-[2-^{3}H] Inositol (100 Ci/mmol) was obtained from Amersham (Les Ulis, France). Dowex (AG1-X8, formate) was purchased from Bio-Rad (Ivry-sur-Seine, France). DMSO and sucrose were purchased from Prolabo (Paris, France). Tris was purchased from Merck-Clevenot (Nogent-sur-Marne, France). SR 46349B was synthesized in the Chemical Department, Sanofi Recherche (Montpellier, France). For *in vitro* binding studies, drugs were dissolved in DMSO. The concentration of DMSO in the assay never exceeded 0.1% (v/v). This final concentration was without effect on radioligand binding.

Animals. CD$_1$ mice (18–20 g; Charles River, France) were used for *in vitro* and *ex vivo* binding studies. Naive male CD$_1$ mice (20–26 g; Charles River) were used to study the behavioral effects of L-5-HTP. Male OF$_1$ mice (18–20 g; Iffa Credo, St. Germain sur Labresle, France) were used for inositol phosphate accumulation studies.

Drug treatments. SR 46349B was dissolved in ethanol and diluted in distilled water as required. Ritanserin was dissolved in tartric acid (15 mg/ml) and diluted in distilled water as required. For acute and chronic treatments an oral bolus dose (10 ml/kg of body weight) of compound was given to groups of mice. Control animals received the vehicle and were kept under the same conditions as the drug-treated animals. Doses are expressed as amounts of the salts. L-5-HTP was dissolved in 1 N HCl, diluted with distilled water, and administered intraperitoneally (200 mg/kg).

Preparation of cerebral cortical membranes. Microsomal membranes were prepared essentially as described by Leysen *et al.* (12). Mice were killed by decapitation. Brains were immediately removed and prefrontal cortex was dissected, pooled, placed in 10 volumes (w/v) of ice-cold buffer A (0.25 M sucrose, 0.05 M Tris·HCl, pH 7.7, 1 mM PMSF, 1 mM DTT), homogenized with a Teflon homogenizer, and centrifuged at 45,000 × *g* at 4° for 30 min. The pellet was washed once by resuspension with a pipette in 10 volumes (w/v) of buffer B (0.05 M Tris·HCl, pH 7.7, 1 mM PMSF, 1 mM DTT) and was centrifuged twice as described above. The final pellet was homogenized with a Teflon homogenizer in buffer B and stored frozen at −80°, at a final concentration of 117 mg of wet weight of tissue/ml.

Binding assays. [^{3}H]Ketanserin binding assays were performed at 37° for 15 min. Membranes (4.7 mg of wet tissue) were incubated with the indicated concentrations of [^{3}H]ketanserin in 1 ml of buffer A (0.05 M Tris·HCl, pH 7.7). A rapid filtration technique using Whatman GF/B filters (pretreated with 0.3%, w/v, polyethylenimine) and a 48-well filtration apparatus (Brandel) was used to harvest and rinse (three consecutive rinses with 5 ml of cold buffer A containing 0.25% bovine serum albumin) the labeled membranes. The radioactivity bound to the filters was counted with 4 ml of Biofluor liquid scintillant. Nonspecific binding was determined in the presence of 1 μM ketanserin.

Measurement of inositol monophosphate accumulation *in vivo*. Groups of six mice were administered SR 46349B (10 mg/kg) or its vehicle orally twice per day (9.00 a.m. and 5.00 p.m.) for 3 days and on the morning of the fourth day. [^{3}H]Inositol monophosphate accumulation studies were carried out essentially as described by Hide *et al.* (13). Two microliters of *myo*-[2-^{3}H]inositol (2 μCi) were injected intracerebroventricularly into mice 8 hr after the last administration. Sixteen hours later, mice were killed by microwave application (3 kW, 1.6 sec). Brains were quickly removed, homogenized with 10 ml of chloroform/methanol/HCl (2:1:0.012, by volume), extracted with 3 ml of water, and centrifuged at 1000 × *g* for 10 min at 4°. The aqueous phases, containing ^{3}H-labeled inositol phosphate, were taken for analysis after chromatography over Dowex-1 ion exchange columns according to the method of Berridge *et al.* (14). Aliquots (1 ml) were withdrawn and the radioactivity was determined by liquid scintillation counting. Except for basal [^{3}H]inositol phosphate level determinations, lithium chloride (10 mEq/kg) was administered intraperitoneally to all animals 2 hr before they were killed. (±)-DOI-treated animals received a single intraperitoneal administration of 1 mg/kg 30 min before they were killed.

5-HTP-induced head-twitches in mice. Groups of 16–20 mice were administered SR 46349B or its vehicle orally, acutely or twice per day (9.00 a.m. and 5.00 p.m.) for 3 days and on the morning of the fourth day. L-5-HTP (200 mg/kg) was injected intraperitoneally 24 or 48 hr after the last treatment, in independent experiments. Another experiment was performed with the same treatment schedule, except that animals (groups of 20 mice) received ritanserin (1 mg/kg orally, or its vehicle) 60 min before L-5-HTP administration. The number of head-twitches occurring in the next 20 min was counted by trained observers in blind conditions (15).

Data analysis. In competition experiments, the drug concentration producing 50% inhibition (IC_{50}) of radioligand binding and the Hill coefficient (n_H) values were determined from Hill plots of log ($B_o - B/B$) versus log (concentration) of test drug, where B_o and B are specific binding in the absence and presence of competitor, respectively. Inhibition constant (K_i) values were calculated from the IC_{50} values by using the Cheng and Prusoff equation (16).

Data from equilibrium binding (K_d and B_{max}) and competition (IC_{50}) experiments were analyzed using a nonlinear least-squares method on a Digital PC 350 computer. All experiments were performed in duplicate and the results were confirmed in at least three independent experiments. Student's t test was used to evaluate significant differences from the control ($p < 0.05$). Data from the L-5-HTP-induced head-twitch response and inositol monophosphate accumulation studies were statistically processed by the Mann-Whitney U test and the Dunnett t test, respectively.

Results

Interaction of SR 46349B with mouse brain 5-HT$_2$ receptor *in vitro*. The ability of ketanserin, ritanserin, and SR 46349B to inhibit mouse brain prefrontal cortical 5-HT$_2$ receptors *in vitro* at 37° is shown in Fig. 2. Ketanserin, ritanserin, and SR 46349B totally inhibited the [^{3}H]ketanserin binding sites in a concentration-dependent manner, with an IC_{50} value of 3.86 ± 0.65 nM (four experiments), 3.34 ± 1.2 nM (three experiments), and 3.52 ± 0.81 nM (four experiments), respectively. The concentration-response curves obtained with unlabeled ketanserin and SR 46349B were compatible with a single-phase competitive model (n_H = 0.98 ± 0.05 and 1.05 ± 0.09, respectively), giving K_i values of 1.60 ± 0.50 nM and 1.64 ± 0.47 nM, respectively. The Hill coefficient value obtained for ritanserin (n_H = 1.27 ± 0.05) suggested a noncompetitive inhibition of the [^{3}H]ketanserin binding by this drug (17).

Saturation binding experiments using [^{3}H]ketanserin were carried out at 37° in the absence (control) or presence of 1 nM or 10 nM SR 46349B or 1 nM ritanserin. As shown in Fig. 3, a significant increase ($p < 0.05$) of the K_d (1 nM SR 46349B, K_d = 1.30 ± 0.15 nM, three experiments versus control, K_d = 0.51 ± 0.03 nM, three experiments) but no significant change ($p > 0.05$) of the B_{max} (1 nM SR 46349B, B_{max} = 12.71 ± 1.14 fmol/mg of cortex, three experiments, versus control, B_{max} = 13.66 ± 0.80 fmol/mg of cortex, three experiments) was observed when SR 46349B was added. Addition of 10 nM SR 46349B

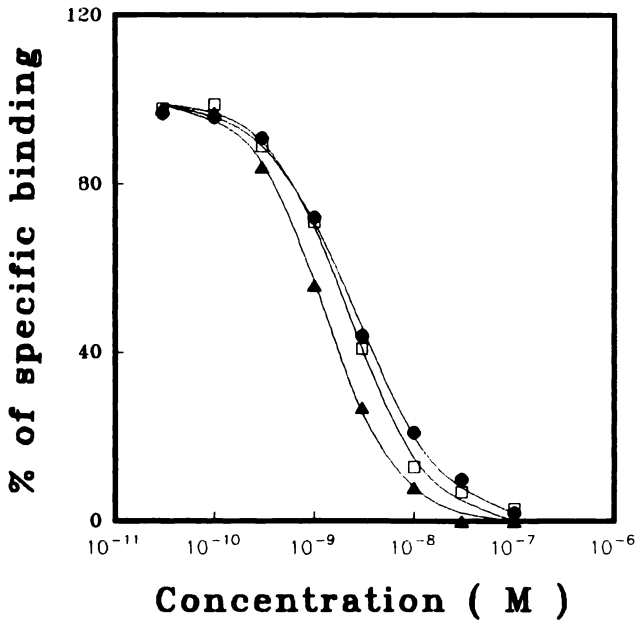

Fig. 2. Inhibition of specific [^{3}H]ketanserin binding to mouse cortical membranes by unlabeled ketanserin (●), ritanserin (▲), and SR 46349B (□). Experiments were carried out at 37° for 15 min using 0.8 nM [^{3}H] ketanserin, as described in Experimental Procedures. Data are from one representative experiment of three performed in duplicate and are expressed as the percentage of specific binding in the absence of competitor.

completely abolished [^{3}H]ketanserin binding to its sites. These results suggested a competitive inhibition of the [^{3}H]ketanserin binding by SR 46349B. Ritanserin at 1 nM reduced significantly ($p < 0.05$) both the affinity (K_d = 1.16 ± 0.47 nM, three experiments) and the maximal binding capacity for [^{3}H]ketanserin (B_{max} = 9.85 ± 1.49 fmol/mg of cortex, three experiments). These results suggested a noncompetitive inhibition of [^{3}H] ketanserin sites by ritanserin.

Effect of chronic and acute treatment with SR 46349B on the [^{3}H]ketanserin binding parameters in mice. The effects of subacute oral treatment (twice per day for 3 days and on the morning of the fourth day, followed by a 1-day drug-free period) on the [^{3}H]ketanserin binding parameters (B_{max}, K_d, and K_i) were investigated. As illustrated in Fig. 4, the B_{max} value of 5-HT$_2$ sites identified with [^{3}H]ketanserin, measured *ex* vivo 24 hr after the last administration, in cortical membranes of treated mice (5 or 10 mg/kg) was significantly increased ($p < 0.05$), by 41% (B_{max} = 17.87 ± 1.08 fmol/mg of cortex, three experiments) and 75% (B_{max} = 22.14 ± 2.28 fmol/mg of cortex, five experiments), respectively, compared with control mice (B_{max} = 12.68 ± 0.81 fmol/mg of cortex, five experiments. No significant difference ($p > 0.05$) in the K_d value was observed between control (K_d = 0.81 ± 0.11 nM) and treated mice (SR 46349B, 5 mg/kg, K_d = 0.82 ± 0.25 nM; SR 46349B, 10 mg/kg, K_d = 1.24 ± 0.21 nM). Moreover, as shown in Table 1, the affinity constant of ketanserin measured *ex vivo* at 1 nM or 10 nM in washed cortical membranes was not changed after chronic treatment with SR 46349B (5 or 10 mg/kg, orally). This effect disappeared 48 hr after the last treat-

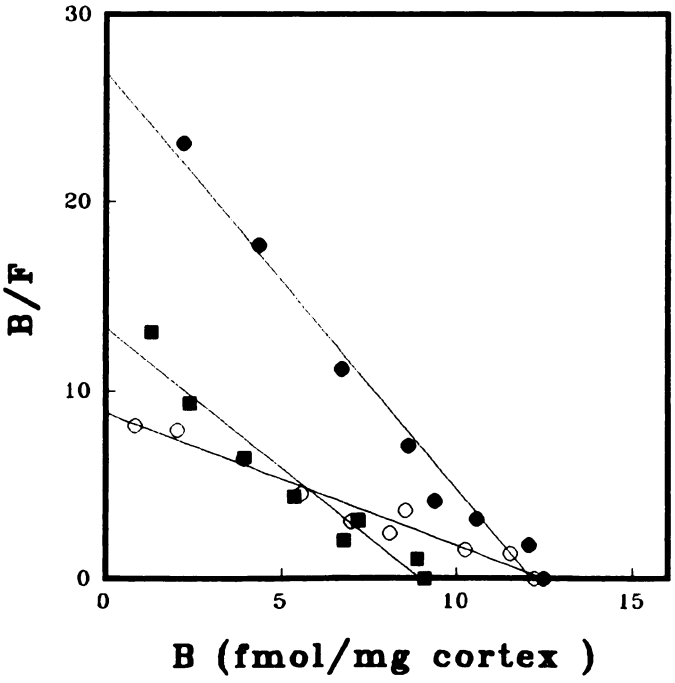

Fig. 3. Effects of SR 46349B on the [^{3}H]ketanserin binding parameters in washed cortical membranes. Equilibrium binding experiments were carried out at 37° for 15 min, as described in Experimental Procedures, with increasing concentrations of [^{3}H]ketanserin ranging from 0.1 to 12 nM in the absence (control) (●) and presence of 1 nM SR 46349B (○) or ritanserin (■). Data for control and drug conditions are from one experiment of three performed in duplicate.

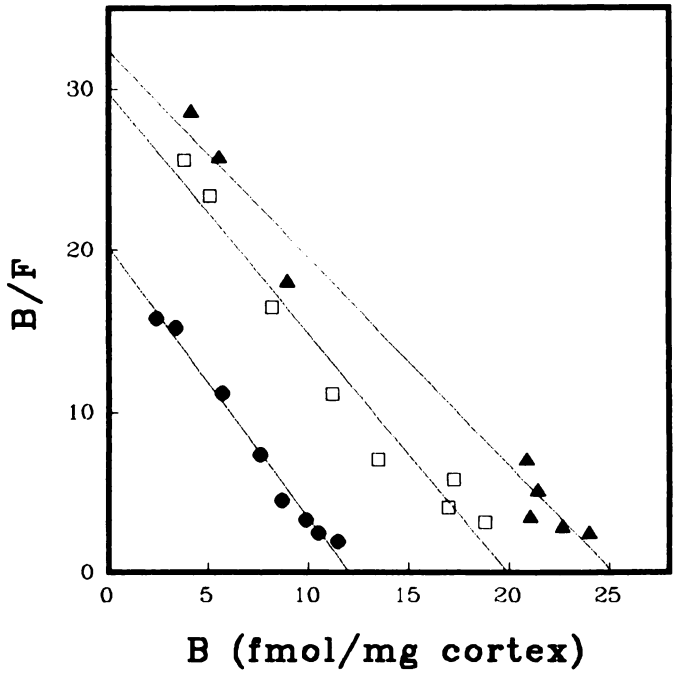

Fig. 4. Effects of chronic treatment with SR 46349B on [^{3}H]ketanserin binding parameters in washed cortical membranes. Mice (13/group) were treated orally with 5 mg/kg (□) or 10 mg/kg (▲) SR 46349B or vehicle (●) twice per day for 3 days and once on the morning of the fourth day and were killed 24 hr later. The specific binding of [^{3}H]ketanserin to 5-HT$_2$ sites was measured as described in Experimental Procedures. The concentration of radioligand ranged from 0.1 to 10 nM. Data for control and drug conditions are from one representative experiment of five performed in duplicate.

TABLE 1

Effect of subacute oral treatment with SR 46349B on the affinity constant of ketanserin

Mice were administered SR 46349B (twice per day for 3 days and once on the morning of the fourth day) and sacrificed 24 hr later. [^{3}H]Ketanserin binding to washed frontal cortical membranes of mice was performed as described in Experimental Procedures, using 1 nM or 10 nM [^{3}H]ketanserin. Values of IC_{50} and n_H are means ± standard deviations from two independent experiments.

	IC_{50}	n_H	K_i
	nM		nM
1 nM [^{3}H]Ketanserin			
Control	2.50 ± 0.15	0.99 ± 0.05	0.74
SR 46349B, 5 mg/kg	2.84 ± 0.46	0.96 ± 0.07	0.84
SR 46349B, 10 mg/kg	2.56 ± 0.20	1.03 ± 0.07	0.76
10 nM [^{3}H]Ketanserin			
Control	13.60 ± 4.90	0.91 ± 0.04	0.55
SR 46349B, 10 mg/kg	15.60 ± 1.60	0.89 ± 0.09	0.63

ment (SR 46349B, 10 mg/kg, B_{max} = 12.40 ± 0.82 fmol/mg of cortex, four experiments). Twenty-four hours after a single oral dose of SR 46349B (10 mg/kg) the B_{max} value of ketanserin was found to be increased by 24% in treated mice (B_{max} = 17.1 fmol/mg of cortex, 95% CL = 15.2–18.0 fmol/mg of cortex), compared with saline-treated mice (B_{max} = 13.8 fmol/mg of cortex, 95% CL = 11.9–15.7 fmol/mg of cortex), whereas the K_d value was not changed (control, K_d = 0.52 nM, 95% CL = 0.35–0.62 nM; SR 46349B, K_d = 0.43 nM, 95% CL = 0.33–0.53 nM). This acute effect disappeared 48 hr after administration (K_d = 0.54 nM, 95% CL = 0.44–0.64 nM; B_{max} = 12.4 fmol/mg of cortex, 95% CL = 11.4–13.3 fmol/mg of cortex).

Effect of chronic administration of SR 46349B on (±)DOI-stimulated inositol phosphoinositide turnover *in vivo*. The effect of chronic oral treatment (twice per day for 3 days plus once per day for 1 day) with SR 46349B (10 mg/kg) on phosphoinositide turnover stimulated *in vivo* by (±)-DOI in mouse brain is illustrated in Fig. 5. In the absence of injection of (±)-DOI there was no significant difference in the [^{3}H] inositol monophosphate levels between control and chronically SR 46349B (10 mg/kg, orally)-treated groups whether the mice had been pretreated with LiCl or not. Intraperitoneal administration of the 5-HT$_2$ agonist (±)-DOI increased the amount of [^{3}H] inositol monophosphate by 24% in the control group and by 65% in the SR 46349B-treated group.

Effect of chronic treatment with SR 46349B on L-5-HTP-induced behavior in mice. The effects of acute and chronic oral treatment with SR 46349B (5 or 10 mg/kg) on L-5-HTP-induced head-twitches in mice are shown in Table 2. It appeared that 24 hr after a single dose of SR 46349B the behavioral response induced by L-5-HTP was not changed. In contrast, after chronic administration (twice per day for 3 days plus once per day for 1 day, followed by a 24-hr drug-free period before the test) of SR 46349B (5 or 10 mg/kg), a 2- or 4-fold increase respectively, of the head-twitch number was found in comparison with vehicle. This effect disappeared 48 hr after the last administration. As shown in Fig. 6, administration of ritanserin (1 mg/kg, orally) 60 min before the L-5-HTP injection completely abolished the head-twitch response in both control and SR 46349B-treated groups. These results pointed out that enhanced behavioral responses induced by repeated administration of SR 46349B were mediated by activation of 5-HT$_2$ receptors.

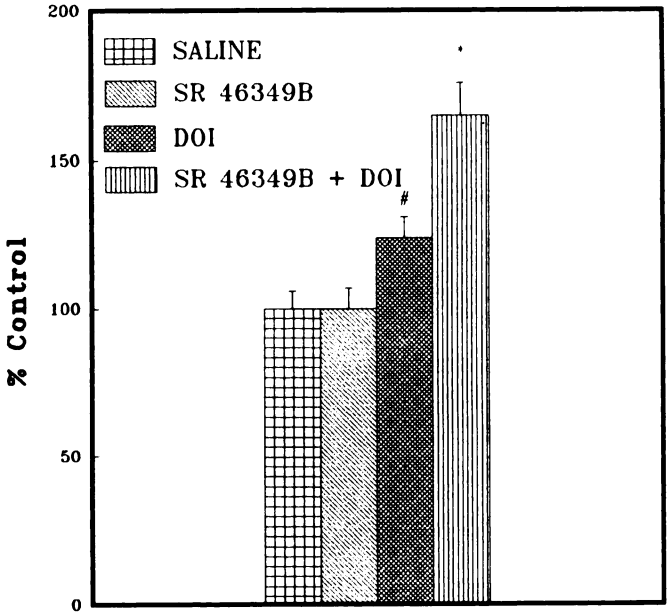

Fig. 5. Effect of subacute treatment with SR 46349B on (±)-DOI-induced [^{3}H]inositol monophosphate accumulation *in vivo*. Animals (6/group) were treated orally with 10 mg/kg SR 46349B or vehicle twice per day for 3 days and once on the morning of the fourth day. Mice were given intracerebroventricular injections of [^{3}H]inositol 8 hr after the last administration. Animals were administered LiCl (10 mEq/kg) 16 hr later and 2 hr before (±)-DOI (1 mg/kg, intraperitoneally) or saline (control) administration. Mice were killed by microwave irradiation 30 min after (±)-DOI or saline injection and [^{3}H]inositol monophosphates from the brain were measured as described in Experimental Procedures. The results are expressed as percentage change (mean ± standard error) in [^{3}H]inositol monophosphate accumulation relative to the saline-treated group, i.e., in the absence of (±)-DOI treatment (100%). Dunnett's *t* test was used to obtain a measure of significance of the differences between saline- and DOI-treated groups (#, $p < 0.05$) and between DOI- and SR 46349B/DOI-treated groups (*, $p < 0.05$).

TABLE 2

Effect of acute or subacute oral treatment with SR 46349B on the L-5-HTP-induced behavioral response in mice, 24 or 48 hr after the last administration

Mice were administered SR 46349B or its vehicle and behavior was determined as described in Experimental Procedures. Values are mean ± standard error.

	Number of head-twitches	
	24 hr	48 hr
Acute treatment		
Control	5.9 ± 1.3	ND[a]
SR 46349B, 5 mg/kg	6.6 ± 1.4	ND[a]
SR 46349B, 10 mg/kg	6.0 ± 2.0	ND[a]
Chronic treatment		
Control	3.8 ± 1.3	6.9 ± 2.6
SR 46349B, 5 mg/kg	9.8 ± 2.7[b]	6.8 ± 1.8
SR 46349B, 10 mg/kg	15.9 ± 4.8[b]	6.9 ± 1.9

[a] ND, not determined.

[b] Significance of difference from control according to Mann-Whitney *U* test, $p < 0.05$.

Discussion

Until now, electroconvulsive shock therapy has been the only treatment known to increase 5HT$_2$ receptor number (18, 19). The present report shows for the first time that repeated administration of a potent competitive and selective 5-HT$_2$ antagonist (11) can also cause an apparent adaptive increase in the density of 5-HT$_2$ sites.

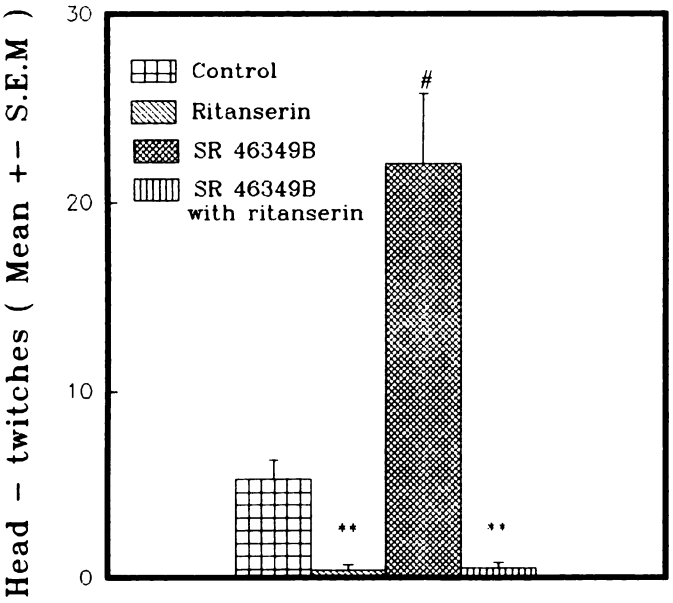

Fig. 6. Effect of ritanserin on enhanced behavioral responses induced by subacute treatment with SR 46349B in mice. Animals (20/group) were treated orally with 10 mg/kg SR 46349B or vehicle twice per day for 3 days and once on the morning of the fourth day. Mice were administered ritanserin (1 mg/kg, orally) or its vehicle 23 hr after the last administration and 1 hr before L-5-HTP (200 mg/kg, intraperitoneally) administration. Behavior was determined as described in Experimental Procedures. Mann-Whitney *U* test was used to obtain a measure of significance of the differences between control and ritanserin-treated groups (**, $p < 0.01$), between SR 46349B- and SR 46349B/ritanserin-treated groups (**, $p < 0.01$), and between control and SR 46349B-treated groups (#, $p < 0.01$).

Investigation of *ex vivo* receptor binding in mouse cerebral cortical membranes 24 hr after chronic oral administration (twice per day for 3 days and on the morning of the fourth day) of SR 46349B (5 or 10 mg/kg) revealed that SR 46349B produced a marked increase of the maximum binding capacity of ketanserin without having any effect on its affinity constant. This effect disappeared 48 hr after the last administration of SR 46349B. Results found after repeated treatment with SR 46349B suggest an up-regulation of 5-HT_2 receptors and differ from all previous observations with other 5-HT_2 receptor antagonists (20).

Because 5-HT_2 receptor stimulation causes inositol phospholipid hydrolysis (21, 22), the effects of chronic administration (twice per day for 3 days and on the morning of the fourth day) of SR 46349B (10 mg/kg) on 5-HT_2 agonist [(±)-DOI]-stimulated phosphoinositide turnover *in vivo* under lithium treatment were investigated. Twenty-four hours after the last administration, a significant increase of the amount of ^{3}H-labeled inositol phosphate was observed in the SR 46349B-treated group, compared with the control group, whereas in the unstimulated group SR 46349B had no effect on the [^{3}H]inositol phosphate levels. This change in the second messenger system correlates with the enhancement of 5-HT_2 receptor number.

Because 5-HT_2 receptors have been implicated in the production of 5-HTP-induced head-twitches in mice (23), the effects of repeated administration of SR 46349B (5 and 10 mg/kg, orally) on this parameter have been tested. A significant increase of the head-twitch response to L-5-HTP was observed 24 hr after stopping of drug treatment, and this returned to normal within 48 hr. Administration of a 5-HT_2 receptor antagonist such as ritanserin 1 hr before the measure of the head-twitch response induced by L-5-HTP completely blocked the behavioral response in both control and SR 46349B-treated groups. This finding confirms that the enhanced response observed 24 hr after subacute administration of SR 46349B is indeed mediated by activation of 5-HT_2 receptors. A transient increase of the 5-HT_2 binding capacity for [^{3}H]ketanserin was also observed 24 hr after a single dose of SR 46349B. However, this increase was only observed with the higher dose of the compound (10 mg/kg, orally). This acute effect is weak and may not be sufficient to cause a significant modification of the behavioral responses induced by L-5-HTP. These results showing a parallel change in 5-HT_2 receptor number, in the signal-transducing system coupled to 5-HT_2 receptors, and in 5-HT_2 receptor-mediated behavior suggest a supersensitivity of 5-HT_2 receptors in mouse brain after chronic treatment with SR 46349B. This adaptive response to SR 46349B treatment appears to be a rapid process and is in accordance with published data relating to the down-regulation observed with 5-HT_2 agonists (4, 6) or antagonists (12). Although the up-regulation observed by repeated administration of SR 46349B is not in accordance with previous reports using 5-HT_2 antagonists, it is the expected response to repeated treatment with an antagonist.

Nevertheless, our results suggest that 5-HT_2 antagonists may up-regulate 5-HT_2 receptors. The difference found between SR 46349B and the well known 5-HT_2 antagonists ketanserin, ritanserin, and mianserin may be ascribed to other properties of these latter drugs. In fact, these compounds are known to bind with high or moderate affinities to other receptors (24), whereas SR 46349B appears to be specific for 5-HT_2 receptors (11). Moreover, the inhibition produced by ritanserin seems to be noncompetitive *in vitro* and *ex vivo*, suggesting an allosteric regulation of 5-HT_2 sites by this compound. Although the lack of selectivity and the type of inhibition exerted by these compounds may account for the unusual adaptive response found with the 5-HT_2 antagonists examined, they do not explain why 5-HT denervation or depletion does not induce up-regulation of 5-HT_2 receptors. In fact, depletion of central stores of serotonin with reserpine (25) or 5,7-dihydroxytryptamine (26) failed to alter binding to 5-HT_2 receptors in the cerebral cortex or hippocampus in rats. However, an enhanced 5-HT_2 behavioral response and receptor number have been reported by others (27), but no change in 5-HT-stimulated phosphoinositide turnover was found (28) after intracerebroventricular administration of 5,7-dihydroxytryptamine in mice. To clarify these discrepancies, SR 46349B can provide (in its tritiated form) a useful tool to explore 5-HT_2 receptor adaptive changes in response to either persistent stimulation (chronic agonist exposure) or blockage (e.g., denervation, depletion, or repeated antagonist treatment). In addition, to elucidate the mechanism of action of SR 46349B, further investigations including *in vivo* regulation of the 5-HT_2 receptor and expression of the corresponding mRNA are going on in rats, after repeated administration of the drug.

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