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Behavioral Profile of the $5HT_{1A}$ Receptor Antagonist (S)-UH-301 in Rodents and Monkeys

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MOREAU, J.-L., G. GRIEBEL, F. JENCK, J. R. MARTIN, U. WIDMER AND W. E. HAEFELY. *Behavioral profile of the $5HT_{1A}$ receptor antagonist (S)-UH-301 in rodents and monkeys.* BRAIN RES BULL **29**(6), 901-904, 1992.—The effects of the new $5HT_{1A}$ receptor antagonist (S)-UH-301 were investigated in several neurological and behavioral tests in rodents and monkeys. By itself, (S)-UH-301 was found to decrease palatable food consumption in rats, to exhibit anticonvulsant activity in mice, and anxiolytic-like properties in two rodent models of anxiety (light-dark test and elevated plus-maze test). (S)-UH-301 antagonized various symptoms and behaviors induced by the selective $5HT_{1A}$ receptor agonist 8-OH-DPAT, such as lower lip retraction and flat body posture in rats, hyperphagia for palatable food in rats, and displacement activities (considered as indices of anxiety) in squirrel monkeys. These results further characterize (S)-UH-301 as an in vivo active $5HT_{1A}$ receptor antagonist and suggest that this antagonistic activity might confer the compound with anxiolytic-like properties.

(S)-UH-301 $5HT_{1A}$ antagonist Anxiety Monkey

SEROTONIN (5HT) is recognized to play a role in anxiety, depression, aggression, and other neuropsychiatric disorders (6). Among the various 5HT receptor subtypes, $5HT_{1A}$ receptors have been shown to be involved in a variety of physiological functions including regulation of sleep, temperature, mood, eating, and sexual behaviors. Their functional role is not yet fully elucidated, possibly due to the complexity of the serotonergic system (functional interactions taking place between different 5HT receptor subtypes) or to the lack of a selective $5HT_{1A}$ receptor antagonist. Recently, a new putative $5HT_{1A}$ receptor antagonist ((S)-5-fluoro-8-hydroxy-2-(dipropylamino)tetralin = (S)-UH-301) has been synthetized (8) and has been shown to antagonize several biochemical, cardiovascular, and behavioral effects induced by the selective $5HT_{1A}$ receptor agonist 8-OH-DPAT (2,3,4,9).

This study describes further effects of (S)-UH-301 in a variety of behavioral tests performed in rodents and monkeys. The focus was on its activity in tests considered predictive of anxiolytic-like activity in these two species.

METHOD

Drugs

8-OH-DPAT (8-hydroxy-2-(dipropylamino)tetralin) and (S)-UH-301 ((S)-5-fluoro-8-hydroxy-2-(dipropylamino)tetralin) were synthetized in the chemical research department at F. Hoffmann-La Roche Ltd. in Basel.

Binding Tests

Affinities for $5HT_{1A}$, $5HT_{1B}$, $5HT_{1C}$, and $5HT_2$ receptors were determined by measuring specific ^{3}H-8-OH-DPAT, ^{3}H-serotonin, ^{3}H-mesulergine, or ^{3}H-ketanserin binding in rat hippocampus, rat cortex, pig choroid plexus, or rat frontal cortex, respectively (see 10,12,18 for more details). The concentrations of the compound which produced half maximal inhibition of these bindings (IC_{50}) were calculated by a nonlinear regression calculation program.

Antagonism of 8-OH-DPAT-Induced Lower Lip Retraction and Flat Body Posture in Rats

8-OH-DPAT has been shown to reliably and specifically produce lower lip retraction and flat body posture in rats (1). In order to assess its antagonistic properties, various doses of (S)-UH-301 were injected IP 30 min before subcutaneous administration of 8-OH-DPAT (0.32 mg/kg). This dose of 8-OH-DPAT was selected as inducing lower lip retraction and flat body posture in more than 90% of the animals. Following treatment, the rats were placed individually in plexiglas cages (30 × 25 × 10 cm) and were scored every 10 min for 1 h beginning 5 min after treatment: 0 = lower incisors not or hardly visible (not different from untreated animals), 0.5 = partly visible, 1 = completely visible. Flat body posture was scored in a similar manner. Eight rats were used per dose. The ID_{10}, ID_{50}, and ID_{90} values were calculated by probit analysis.

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Convulsion Tests in Mice

The anticonvulsant potency and efficacy of (S)-UH-301 in preventing (15 or 30 min pretest IP or SC administration, respectively) tonic convulsions induced either by intracerebroventricular (ICV) injection of N-methyl-D-aspartic acid (NMDA, 1 nmol in 2 μl) or by acoustic stimulation (110 dB) in the genetically seizure-susceptible mouse strain DBA/2J were evaluated. Eight mice were used per dose. ED_{50} values were calculated by probit analysis.

Conflict Test in Rats

An operant anticonflict paradigm was used to evaluate disinhibition of punished responding in Fü-rats. (S)-UH-301 was given subcutaneously or orally 30 min before testing and the minimal effective dose (MED) producing a significant increase in punished responding was determined using the Mann-Whitney U test to make comparisons between vehicle and each individual dose condition. Further methodological details are provided elsewhere (11).

Motor Performance Test in Mice and Rats

The motor impairing effects of (S)-UH-301 (administered SC 30 min prior to the test) were evaluated in mice and rats in a rotarod test. The number of animals (out of eight per dose) remaining for at least 1 min on the rotating rod was recorded. ED_{50} values were calculated by probit analysis.

Palatable Food Consumption in Rats

Adult rats maintained on ad lib diet of standard rat chow were periodically tested (a minimum of 2 days between tests) 30 min after SC administration of vehicle or different doses of (S)-UH-301 to determine their consumption of palatable food (boiled potatoes) during a 30 min session in a plastic test cage (with the dimensions 26 × 20 × 13 cm). When evaluating its antagonistic properties, (S)-UH-301 was administered SC 15 min after 8-OH-DPAT (0.6 mg/kg PO, a dose shown to produce a slight but significant hyperphagic effect). Palatable food intake after each individual dose condition was compared to the mean for several sessions with vehicle using a Wilcoxon test.

Light–Dark Test in Mice

This test is based on the natural tendency of most mouse strains to avoid the aversive properties of a brightly lit environment. The apparatus consisted of two plexiglas boxes (20×20×14 cm), a darkened one connected by an opaque plastic tunnel (5×7×10 cm) to an illuminated one. Thirty min after IP administration, mice were placed in the lit box to start the test session. The amount of time spent in the lit box and the number of transitions through the tunnel were recorded over a 5-min period. Further methodological description can be found elsewhere (7). Statistical significance of differences between control and treated groups was ascertained with a combined analysis of variance (ANOVA) followed by the Bonferroni's t test.

Free Exploratory Test in Mice

A polyvinyl box (30×20×20 cm), subdivided into six equal square units could be temporarily divided into two halves by means of three partitions. Each mouse was familiarized for about 24 h with one half of the apparatus. Approximately 1 day later, and 30 min after IP administration, each mouse was exposed to the entire box and the time spent in the novel compartment, the locomotor activity, and the number of rearing responses were recorded over a 10-min period (for more details, see 7).

Elevated Plus-Maze in Rats

The apparatus was 50 cm above the floor and consisted of two open arms (50×10 cm) perpendicular to two enclosed arms (50×10×40 cm) extending from a central platform (10×10 cm). Thirty minutes after IP injection, the rat was placed in the center of the plus maze facing one of the closed arms. During the 5-min test period, the following video measurements were taken by an observer: the number of entries into open and into closed arms, the time spent in open arms, and the number of times the animal reared. A rat was considered to have entered an arm when all four paws were in the arm. The test was performed and the data analyzed as described elsewhere (13).

Free Behavior Observation in Monkeys

The compound was orally administered to squirrel monkeys which were then observed periodically for a number of behavioral alterations by a trained observer during a period of at least 6.5 h. When evaluating its antagonistic properties, (S)-UH-301 was given orally 15 min before a SC administration of 8-OH-DPAT (0.1 mg/kg). This dose of 8-OH-DPAT was selected as the lowest to reliably induce both scratching behavior and refusal of food (considered as behavioral indices of anxiety in nonhuman primates, 17) in the majority of animals. These symptoms were scored for 2 h following treatment.

RESULTS

(S)-UH-301 was found to displace with moderate affinity but selectively the binding to the $5HT_{1A}$ recognition site (IC_{50} = 98 nM) as compared to the $5HT_{1B}$ (IC_{50} > 100 μM), $5HT_{1C}$ (IC_{50} = 7150 nM) and the $5HT_2$ (IC_{50} = 7200 nM) receptor subtypes.

Effects of (S)-UH-301 Alone

As shown in Table 1, following SC or IP injections, (S)-UH-301 exhibited dose-dependent anticonvulsant activity in two different mouse models of epilepsy, dose-dependent hypophagic effects in a palatable food consumption test in rats, and anxiolytic-like activity in two unconditioned conflict tests (the light/dark test in mice and the elevated plus-maze test in rats) but not in the conditioned conflict test in rats. Motor disturbances or sedative effects are unlikely to contribute to this anxiolytic-like activity as the active doses were shown not to affect rotarod performances in rats and mice and free exploration in mice. At the highest dose tested (30 mg/kg), (S)-UH-301 slightly increased locomotion in mice and rats which resulted in an impairment of rotarod performances in half of the animals. In squirrel monkeys, doses up to 10 mg/kg produced no detectable effects whereas at the dose of 30 mg/kg, signs of muscle relaxation which were qualitatively scored as weak could occasionally be observed.

Antagonism of 8-OH-DPAT-Induced Behavioral Effects

The involvement of $5HT_{1A}$ receptors in the functional effects induced by (S)-UH-301 was confirmed and further characterized by the following observations (see Table 1): (S)-UH-301 was able to dose-dependently antagonize 8-OH-DPAT-induced lower lip retraction and flat body posture in rats with ID_{50} values of 6.3 and 3.8 mg/kg, respectively and to inhibit 8-OH-DPAT-induced hyperphagic effects in rats. When administered orally in monkeys, it also dose-dependently antagonized 8-OH-DPAT-

TABLE 1

BEHAVIORAL PROFILE OF (S)-UH-301 IN A VARIETY OF TESTS IN RODENTS AND MONKEYS

Effects of the compound alone	
Prevention of audiogenic seizures in mice (mg/kg, SC)	ED_{50} = 11.3 (ED_{10} = 6.3, ED_{90} = 20.3)
Prevention of NMDA-induced seizures in mice (mg/kg, SC)	ED_{50} = 1.4 (ED_{10} = 0.02, ED_{90} = 99.8)
Palatable food intake in rats (mg/kg, SC)	1: inactive; 3–10: decreased intake
Anti-conflict activity in rats (mg/kg, SC)	0.3–30: inactive
Light-dark test in mice (mg/kg, IP)	one dose active: 1
Elevated plus-maze in rats (mg/kg, IP)	one dose active: 1
Free exploration test in mice (mg/kg, IP)	0.3–10: inactive; 30: reduction
Rotarod performance in mice (mg/kg, SC)	1–10: inactive; 30: slight impairment
Rotarod performance in rats (mg/kg, SC)	3–10: inactive; 30: slight impairment
Free behavior in monkeys (mg/kg, PO)	1–10: inactive; 30: muscle relaxation
Antagonistic effects	
Antagonism of DPAT-induced LLR in rats (mg/kg, IP)	ID_{50} = 6.3 (ID_{10} = 1.7, ID_{90} = 23)
Antagonism of DPAT-induced FBP in rats (mg/kg, IP)	ID_{50} = 3.8 (ID_{10} = 0.6, ID_{90} = 25)
Antagonism of DPAT-induced hyperphagia in rat (mg/kg, SC)	0.6: active
Antagonism of DPAT-induced scratching in monkeys	10–30 mg/kg PO: active
Antagonism of DPAT-induced food refusal in monkeys	10–30 mg/kg PO: active

Abbreviations: DPAT: 8-OH-DPAT; FBP: flat body posture; LLR: lower lip retraction. Active means $p < 0.05$ at least, in the various statistical tests.

induced scratching bouts (Fig. 1) and food refusal (two symptoms thought to reflect manifestation of anxiety in nonhuman primates).

DISCUSSION

The present results establish that (S)-UH-301 is a selective ligand at the $5HT_{1A}$ receptor subtype with at least 70-fold greater selectivity for this recognition site over $5HT_{1B}$, $5HT_{1C}$, and $5HT_2$ receptor subtypes. When administered alone, (S)-UH-301 was found to exhibit anticonvulsant, hypophagic, and anxiolytic-like activities. These properties might result from the antagonism of some tonically active $5HT_{1A}$ systems involved in the regulation of mood and eating behaviors. However, at the present time it is not possible to completely exclude a contribution of other receptors. Indeed, affinity of the compound to many of the known receptors has yet to be determined. (S)-UH-301 has been found to weakly bind to the dopamine D2 receptor, but the affinity was only about one-tenth of that for the $5HT_{1A}$ receptor (8) and there is some evidence that the $5HT_{1A}$ antagonistic effects of (S)-UH-301 are not mediated via dopamine receptors (2).

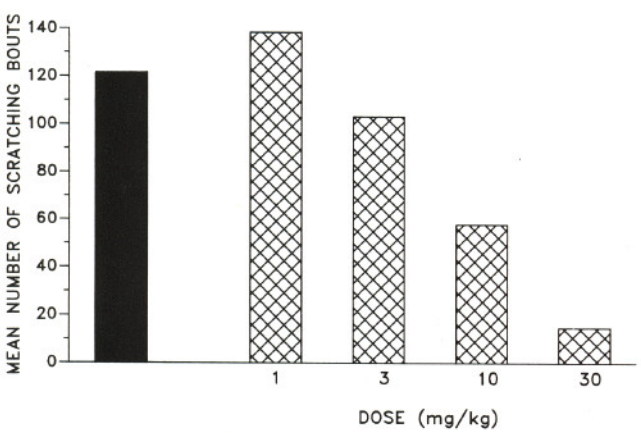

FIG. 1. Antagonism of 8-OH-DPAT-induced scratching behavior in squirrel monkeys. (S)-UH-301 (cross-hatched bars) or vehicle (solid bar) was orally administered 15 min prior to SC administration of 8-OH-DPAT (0.1 mg/kg). The number of scratching bouts occurring during the next 2 h was recorded. Scratching behavior in control animals (not injected with 8-OH-DPAT, data not shown) was barely observable (less than 15 bouts in the 2-h observation period).

Behavioral studies in rodents and monkeys further characterize this compound as a functional $5HT_{1A}$ receptor antagonist. (S)-UH-301 was able to antagonize a number of behaviors induced by 8-OH-DPAT, such as lower lip retraction and flat body posture (symptoms considered to reflect selective activation of pre- and postsynaptic $5HT_{1A}$ receptors, respectively) (1,16) or hyperphagia. In squirrel monkeys, it antagonized 8-OH-DPAT-induced scratching, head shakes (data not shown), and food refusal. Monkeys tend to display these types of behaviors apparently out of context when they experience situations of impending aggression, danger, conflict, tension, or uncertainty (14). These behaviors, referred to as displacement activities in the ethological literature (5), seem to show a resemblance to clinical anxiety which is in some respect superior to that of conflict paradigms in rodents. Indeed, a recent study has shown that scratching could be used as a behavioral measure in studies investigating nonhuman primate models of anxiety (15). Therefore, these results suggest that (S)-UH-301 might also exhibit anxiolytic-like properties in monkeys.

The major finding of this study is that (S)-UH-301, a selective orally active $5HT_{1A}$ antagonist, exhibits anxiolytic-like effects in some tests in mice, rats, and monkeys. Therefore, these data shed more lights on the consequences of inhibition of $5HT_{1A}$ receptors and suggest that the reported anxiolytic-like effects of $5HT_{1A}$ ligands such as buspirone or ipsapirone are likely due to their antagonistic efficacy at the $5HT_{1A}$ receptor.

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