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Anxiolytic-like effects of a selective 5-HT_{1A} agonist, S20244, and its enantiomers in mice

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THE action of a selective 5-HT_{1A} agonist S20244 and its two enantiomers (+)-S20499 and (−)-S20500 were assessed in mice. The animals were confronted with a free exploratory test especially adapted to reveal behavioural sedation, and with a two-box light/dark choice situation validated for the detection of anti-anxiety agents. These drugs were found to have anxiolytic properties at low doses, like benzodiazepines. Furthermore, the drugs exhibited sedative effects at higher doses. These results closely resemble those we found after administration of two other 5-HT_{1A} agonists, 8-OH-DPAT and MDL 73005EF (*NeuroReport*, 1, 267–270, 1990).

Key words: 5-HT_{1A} receptor agonists; Anxiety; Light/dark choice test; Mice

Introduction

The existence of multiple types of 5-HT receptors has led to suggestions that compounds with selective affinity for 5-HT_{1A} binding sites in the CNS may have among other activities special anti-anxiety properties in psychiatric treatment. The recent introduction of buspirone as a drug effective in treating anxiety[1] and the discovery that this drug and other azapirones such as ipsapirone and gepirone possess high affinity for the 5-HT_{1A} subtype of central 5-HT receptors[2] have suggested that these receptors could be strongly implicated in the anxiolytic action of these drugs. Although the most anxiolytic-like effects of 5-HT_{1A}-selective drugs have been demonstrated in pigeons,[3–5] the 5-HT_{1A} agonist 8-OH-DPAT and 5-HT_{1A}-selective azapirones were also able to increase inhibited responding in rats,[6–12] in mice[13] and in primates.[14]

The present investigations examined the potential anxiolytic-like effects of a new 5-HT_{1A} agonist, S20244 (4-*N*-[5-methoxychromane-3-yl] *N*-propylamino butyl-8-azaspiro 4,5 decane-7,9 dione, hydrochloride) and its two enantiomers, (+)-S20499 and (−)-S20500, using an unconditioned conflict test, the light/dark choice procedure, behaviourally validated for detecting anti-anxiety agents in mice.[15] However, since a behavioural sedation has been observed after administration of other 5-HT_{1A} agonists at high doses,[13] a first experiment was undertaken in order to detect possible sedative effects, using a free exploratory test which allows measurement of changes in novelty-seeking behaviour as well as in locomotor and rearing activities. Binding studies indicated that S20244, (+)-S20499 and (−)-S20500 bound selectively to 5-HT_{1A} receptors with K_i values of 0.60 nM, 0.30 nM and 2.0 nM respectively, and acted at these sites as full agonists.[16]

Materials and Methods

Animals: Male Swiss albino mice ('Centre d'Elevage R. Janvier' and the 'Centre d'Elevage Iffa-Credo,' France), 12 weeks of age at time of testing, were used. Prior to experimental testing, they were housed five to a standard cage containing a supply of food pellets and water, and kept on a 12/12 h light/dark cycle with lights on at 1 a.m. in order to observe animals in their high activity period, that is, when the lights are off. In all experimental procedures, each mouse was only tested once.

Drugs: S20244, (+)-S20499 and (−)-S20500 (Servier) were dissolved in saline with a drop of Tween 80 and administered i.p., 20 min before testing, in concentrations giving an injection volume of 10 ml kg^{-1} body wt.

Statistical analysis: Statistical significance of differences between control and treated groups was ascertained by a combined analysis of variance and a Dunnett's (equality of means are assumed to be equal) or Bonferroni's (variances are not assumed to be equal) posteriori *t*-test.

Experiment 1

Apparatus: The apparatus consisted of polyvinylchloride box (30 × 20 × 20 cm) covered with plexiglass and subdivided into six equal square exploratory units, which were all interconnected by small doors. It could be divided in half lengthwise by closing three temporary partitions. The apparatus was kept on a stand in the mouse room. The experimenter stood next to the box always at the same place.

Procedure: Approximately 24 h before testing, each subject was placed in one half of the apparatus with the temporary partitions in place, in order to be familiarized with it. The floor of this half was covered with

sawdust and the animal was given unlimited access to food and water. Next day, the subject was exposed to both familiar and novel compartments by removal of the temporary partitions. It was then observed, in red light, for 10 min. The time spent in the novel half (novelty preference), the number of units entered (locomotion) and the number of rears made by the animals were recorded.

Mice were randomly allocated into the following groups: (a) S20244: vehicle control (saline; $n = 10$) and drug (2, 4, 8 and 16 mg kg^{-1} in saline; $n = 10$); (b) (+)−S20499: vehicle control (saline); $n = 10$) and drug (2, 4, 8 and 16 mg kg^{-1} in saline; $n = 10$); (c) (−)-S20500: vehicle control (saline; $n = 10$) and drug (2, 4, 8 and 16 mg kg^{-1} in saline; $n = 10$).

Experiment 2

Apparatus: The apparatus consisted of two polyvinylchloride boxes (20 × 20 × 14 cm) covered with plexiglass. One of these boxes was darkened. A light from a 100 w desk lamp, 25 cm above the other box provided the only room illumination. An opaque plastic tunnel (5 × 7 × 10 cm) separated the dark box from the lit one. During observation, the experimenter sat in the same place, next to the apparatus.

Procedure: The subjects were individually tested in 5 min sessions in the apparatus described above. The floor of the boxes was cleaned between test sessions. Testing was performed between 2 p.m. and 4 p.m. Mice were placed in the lit box to start the test session. The amount of time spent by mice in the lit box (TLB) and the number of transitions through the tunnel were recorded over a 5 min period, after the first entry in the dark box. A mouse whose four paws were in the new box was considered as having changed boxes.

Mice were randomly divided into the following groups: (a) S20244: vehicle control (saline; $n = 30$) and drug (1, 2 and 3 mg kg^{-1} in saline; $n = 19$); (b) (+)-S20499 and (−)-S20500: vehicle control (saline; $n = 24$) and drugs [(+)-S20499: 1, 2 and 3 mg kg^{-1} in saline; (−)−S20500: 2, 3 and 4 mg kg^{-1} in saline; $n = 12$]. The latter dose range was determined by the marked behavioural disorganization observed at high doses in experiment 1.

Results

Experiment 1: Analysis of variance revealed significant differences among groups for novelty preference in mice treated with (+)−S20499 ($p < 0.001$), but not in mice treated with S20244 and (−)-S20500, for the locomotion in mice treated with S20244, (+)-S20499 and (−)-S20500 ($p < 0.001$) and for rearing behaviour in mice treated with S20244, (+)-S20499 and (−)-S20500 ($p < 0.001$). Figure 1 shows that S20244 significantly decreased locomotion and rearing behaviour at the highest doses (8 and 16 mg kg^{-1}). (+)-S20499 significantly decreased novelty preference at the highest dose (16 mg kg^{-1}), locomotion and rearings at 4, 8 and 16 mg kg^{-1}. (−)-S20500 significantly decreased locomotion at the highest dose (16 mg kg^{-1}) and rearing behaviour at 8 and 16 mg kg^{-1}.

Experiment 2: In mice treated with S20244, analysis of variance revealed significant group differences for the time spent by mice in the lit box ($p < 0.002$) as well as for the number of transitions ($p < 0.01$). Figure 2 shows that this drug at 2 mg kg^{-1} significantly increased both parameters. In mice treated with (+)-S20499 and (−)-S20500, ANOVA revealed significant group differences for the time spent by animals in the lit box ($p < 0.003$) and for the number of transitions

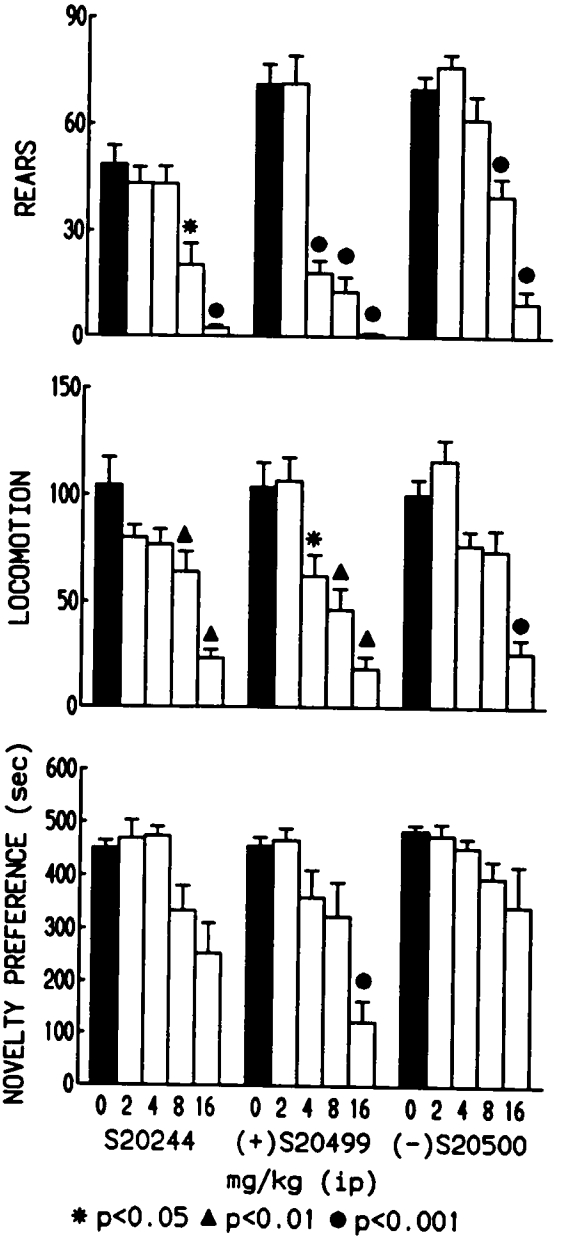

FIG. 1. Effects of S20244, (+)-S20499 and (−)-S20500 on the behaviour of mice confronted with the free exploratory test (means ± s.e.m.). These drugs induced a dose-depended decrease in time spent by mice in a novel compartment (novelty preference) as well as in locomotion and rears (behavioural sedation).

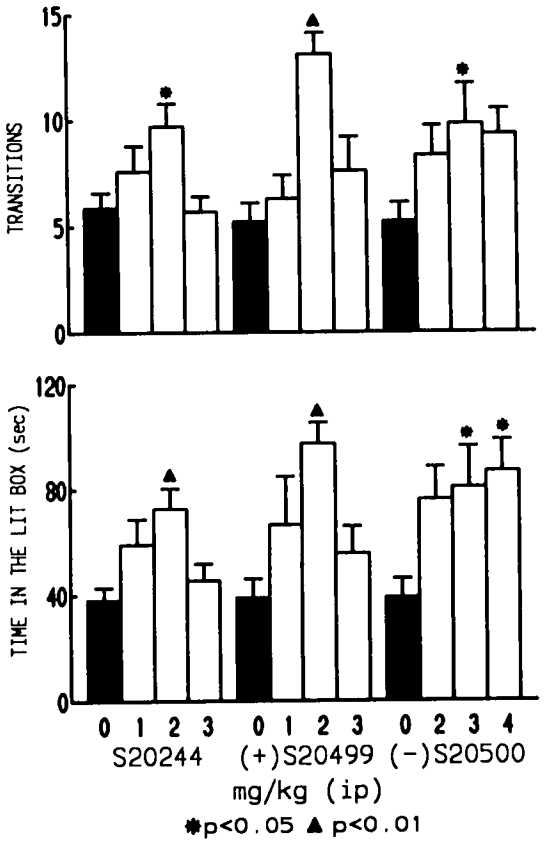

FIG. 2. Effects of S20244, (+)-S20499 and (−)-S20500 on the time spent by mice in the lit box as well as the number of transitions between the boxes (means ± s.e.m.). The drugs significantly increased the time spent by mice in the lit box as well as the number of transitions between the two boxes (anxiolytic-like effects).

($p < 0.001$). Figure 2 shows that (+)-S20499 significantly increased both behavioural parameters at 2 mg kg^{-1} while (−)-S20500 exhibited the same effects at higher doses (3 and 4 mg kg^{-1}).

Discussion

The present results clearly demonstrate that the full 5-HT$_{1A}$ receptor agonists S20244, (+)-S20499 and (−)-S20500 induced at doses which were devoid of sedative properties anxiolytic-like effects insofar as they increased in mice confronted with the light/dark choice procedure the time spent by animals in the lit box as well as the number of transitions between the two boxes. These effects closely resemble those observed in the same situation by Belzung *et al*[17] using a benzodiazepine. These results also confirm and extend previous reports of anxiolytic-like activity in the same test in mice after administration of two other 5-HT$_{1A}$ receptor agonists, 8-OH-DPAT and MDL 73005EF.[13] Moreover, it must be noted that when compared as to their anti-anxiety effects. (+)-S20499 seems to be slightly more active than the other drugs, since it was effective at 2 mg kg^{-1} while the others became effective only at higher doses. These effects could be related to the highest affinity of (+)-S20499 for 5-HT$_{1A}$ receptors in the rat brain as well as to the strongest inhibition induced by this enantiomer of the firing of the dorsal raphé nucleus.[16] Since 5-HT$_{1A}$ receptor agonists such as buspirone and ipsapiron[18–20] as well as 8-OH-DPAT[20] are known to also reduce raphé cell firing, the present results provide additional evidence that the anxiolytic effects of these compounds could be partially mediated by cell body 5-HT$_{1A}$ receptor activation insofar as these receptors were found to be more sensitive than those located postsynaptically.[21,22] Finally, it is to be noted that the drugs used here induced only at high doses, like 8-OH-DPAT and MDL 73005EF, behavioural sedation in a free exploratory test.

Conclusion

The present results show that the 5-HT$_{1A}$ agonist, S20244, and its enantiomers possess the same pharmacological profile (i.e. anxiolytic effects like benzodiazepines) as those we observed with other 5-HT$_{1A}$ agonists such as 8-OH-DPAT and MDL 73005EF.

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