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Effects of S-21007, a potent 5-HT_3 partial agonist, in mouse anxiety

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KEY WORDS S-21007; serotonin agonists; anxiety; animal behavior; maze learning; motor activity; exploratory behavior; choice behavior

ABSTRACT

AIM: To study the effect of S-21007, a 5-HT_3 partial agonist in different animal models of anxiety in mice. **METHODS**: S-21007 effects were evaluated in the behavior tests after intraperitioneal and oral acute treatment or in the light/dark test after both acute and chronic treatments. **RESULTS**: S-21007 presented anxiolytic-like properties after acute administration in the light/dark box test, the mirrored chamber test, and the elevated plus-maze at low doses 10 $ng \cdot kg^{-1}$ – 100 $\mu g \cdot kg^{-1}$, 1 – 100 $\mu g \cdot kg^{-1}$ and 10 – 100 $\mu g \cdot kg^{-1}$, respectively. In the light/dark box test, S-21007 was active orally after acute treatment at 100 $ng \cdot kg^{-1}$ – 10 $mg \cdot kg^{-1}$ and after chronic treatment (14 d) at 1 – 10 $\mu g \cdot kg^{-1}$. S-21007 was devoid of sedative or stimulatory effects. **CONCLUSION**: S-21007 exhibited anxiolytic-like properties. The mechanism of action may be a desensitization of 5-HT_3 receptor or an antagonist activity on the 5-HT_3 receptors.

INTRODUCTION

Several behavioral studies using 5-HT_3 ligands have been reported since few years. Most of them referred to the antiaversive properties of 5-HT_3 antagonists[1]. The lack of psychobehavioral studies concerning the 5-HT_3 agonists was mainly due to their inability to cross the blood-brain barrier. Recently a novel potent 5-HT_3 agonist, SR 57227A, which crossed the blood brain barrier has been presented as active in different antidepressant tests, including the behavioral despair and the learned helplessness models[2].

S-21007, 5-(4-benzyl piperazin-1-yl)-4*H*-pyrrolo [1,2-a]thieno [3,2-e]pyrazine, is a potent and selective 5-HT_3 ligand[3] which has been characterized as a partial agonist in different *in vitro* and *in vivo* models[4]. S-21007 increased [^{14}C]guanidinium uptake in NG 108-15 cells exposed to substance P. This uptake has been described as a reliable test for assessing the functional state of 5-HT_3 receptors in NG 108-15 cells and to characterize the 5-HT_3 compounds. The agonistic effect of S-21007 in this model was clearly antagonized by the 5-HT_3 antagonist, ondansetron. Moreover S-21007 (iv) was a partial agonist in the Von Bezold Jarisch test in anesthetised rat. S-21007 (IC_{50}: 27 $\mu mol \cdot L^{-1}$) was able to induce a 5-HT current in patch clamp experiment on N1E 115 cell line as 5-HT or 5-HT_3 agonists but antagonized this same current at 1 $\mu mol \cdot L^{-1}$. These results lead to the conclusion that S-21007 was a partial agonist which could antagonize 5-HT effects at the 5-HT_3 receptor level by blocking these receptors in a desensitized state. Preliminary tests have shown that this compound induces sedation and decreases reactivity at high doses (128 $mg \cdot kg^{-1}$, ip) in mice. These two central effects have been taken as a sufficient indirect proof of the passage of the blood-brain barrier by at least a small quantity of product and has justified the studies of this 5-HT_3 partial agonist in different animal models of anxiety.

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To select doses devoid of sedative or stimulatory effects, the activity of S-21007 was firstly investigated using a locomotor activity test and a free exploratory test which allows measurement of changes in novelty-seeking behavior as well as in locomotor and rearing activities. S-21007 was after studied in different models validated to detect compounds with anxiolytic properties such as the light/dark choice procedure, the mirrored chamber test and the elevated plus maze test[5,6].

MATERIALS AND METHODS

Mice Two different mouse strains were used in these different experiments. For the locomotor activity, the exploratory behavior and the light/dark box tests, male Swiss mice (Centre d'élevage IFFA CREDO, France) 10 weeks of age were used. For the Mirrored Chamber and the elevated plus maze tests, male BALB/c BryJ inbred mice (The Jakson Laboratory), 8 to 10 weeks of age were studied. The two strains were housed in the same conditions, five per cage under controlled environmental conditions with a 12 h light/dark cycle and *ad libitum* access to food and water. In all experimental procedures, each mouse was only tested once.

Drug S-21007 was dissolved in physiological (0.9 %) saline, which, alone, served for control injections. All administrations were performed in a volume of 10 mL·kg^{-1}.

Locomotor activity over a 90-min period The apparatus consisted of two polyvinyl chloride boxes (20 cm × 20 cm × 14 cm) covered with Plexiglas. An opaque plastic tunnel (5 cm × 7 cm × 10 cm) separated one box from the other. Each box was equipped with a infrared photocell. The beams of photocells permitted an automatic recording of transitions between the two boxes. Experiments were performed under red light. Testing was carried out between 9 : 00 and 18:00. Mice were placed in one of the boxes to start the test session immediately after an ip injection of either saline or S-21007. Number of transitions between the two boxes were recorded over a 90-min period. Floors of boxes were thoroughly cleaned after removal of each mouse. Mice were randomly divided into following groups: vehicle control (n = 13), 1 μg·kg^{-1}, 10 μg·kg^{-1}, 100 μg·kg^{-1}, 1 and 10 mg·kg^{-1} (n = 9). Data were subjected to a two-factor (ANOVA), Group × Time, with repeated measures. Newman-Keuls analysis was used post-hoc to compare treatment groups with the vehicle control group.

Exploratory behavior The apparatus consisted of a polyvinylchloride box (30 cm × 20 cm × 20 cm) covered with Plexiglas and subdivided into six equal exploratory units, which were all interconnected by small doors. It could be divided in half lengthwise by closing three temporary partitions. The experimenter always stood next to the box in the same place. Approximately 24 h before testing, each subject was randomly 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 only was covered with fresh sawdust and the animal was given unlimited access to food and water during the familiarization phase. The duration of this period was 24 h. On the test day, the temporary partitions between the familiar and novel compartments were removed, and the subject was then observed, under red light, for 10 min. Measures of the time spent in the novel half (novelty preference), the number of units entered (locomotion), and the frequency of rears made by the animals (rears) were recorded. Subjects were randomly allocated to one of five treatment groups: saline (n = 10), 1 μg·kg^{-1} (n = 9), 10 μg·kg^{-1}, 100 μg·kg^{-1} and 1 mg·kg^{-1} (n = 10). The data were analyzed by ANOVA. Subsequent comparisons between treatment groups were carried out using Bonferroni procedures. Mice were ip administered 30 min before experiments were carried out.

Light/dark choice paradigm, single acute injection The apparatus consisted of two polyvinylchloride boxes (20 cm × 20 cm × 14 cm) covered with Plexiglas. One of these boxes was darkened. A light from a 100-W desk lamp, 25 cm above the other box provided the room illumination. The light intensity on the center of the illuminated box was approximately 4000 lux. An opaque plastic tunnel (5 cm × 7 cm × 10 cm) separated the dark box from the illuminated one. The subjects were individually tested in 5-min sessions. The floor of each box was cleaned between test sessions. Testing was performed between 14:00 and 17:00. At the start of the test session mice were placed in the tunnel, facing the dark box. The amount of time spent by mice in the lit box (TLB) and

the number of transitions through the tunnel were recorded after the first entry in the dark box. A mouse whose four paws were in the new box was considered as having changed boxes. In the first experiment, mice were injected ip 30 min before testing was carried out. Subjects were randomly allocated to one of six treatment groups: saline ($n = 36$), 10 ng·kg^{-1}, 100 ng·kg^{-1}, 1 μg·kg^{-1}, 10 μg·kg^{-1} and 100 μg·kg^{-1} ($n = 30$). In the second experiment, mice were injected orally 30 min before testing was carried out. Subjects were randomly allocated to one of eight treatment groups: saline ($n = 40$), 10 ng·kg^{-1}, 100 ng·kg^{-1}, 1 μg·kg^{-1}, 10 μg·kg^{-1}, 100 μg·kg^{-1}, 1 and 10 mg·kg^{-1} ($n = 30$). Comparisons between groups were made using a combined ANOVA followed by a Bonferroni's *a posteriori* *t*-test.

Light/dark choice paradigm, chronic treatment Mice were assigned randomly to treatment with either saline (vehicle control, $n = 15$) or 10 ng·kg^{-1}, 1 μg·kg^{-1} and 100 μg·kg^{-1} ($n = 15$) of S-21007. These doses were chosen because acute treatment experiment revealed that they produced reliable anxiolytic-like effects. Subjects received a daily oral dose of either saline or S-21007 during 14 d. Twenty-four hours after the last injection, and 30 min before testing was carried out, vehicle control group was challenged with an acute saline dose and drug groups with an acute S-21007 dose. Comparisons between groups were made using a combined ANOVA followed by a Bonferroni's *a posteriori* *t*-test (for more details, see preceding experiment).

Mirrored chamber test The apparatus used to determine mirror aversion behavior has been described in detail[5]. It consisted of a mirrored cubical chamber (30 cm × 30 cm × 30 cm), constructed from 5 pieces of one-sided mirrored glass, placed inside a rectangular Plexiglas container (40 cm × 40 cm × 40 cm). The mirrored surfaces were located on the interior of the cube. The container had a white floor and opaque black walls. The open side of the mirrored chamber faced a mirror on the container wall. Except for this one mirrored portion, all container walls were black. Behavioral evaluations were carried out in a quiet room under fluorescent lighting. Luminance in the corridor surrounding the mirrored chamber was 200 lux. Light intensity within the mirrored chamber itself was 100 lux.

To begin the assessment of aversion behavior, group housed mice were brought in their home cage into the room where the experiment was conducted and were allowed to accommodate to the new environment for 30 min prior to initiation of the experiment. Mice were exposed to the mirrored chamber and evaluated only a single time to avoid habituation problems. They had experienced no significant handling nor had they been exposed to other behavioral apparatus prior to this use. Changing of the litter in the animal's home cage on the day of the experiment was avoided because it was found to disrupt this behavioral assay.

The behavioral evaluation was begun by placing a single, previously injected mouse at a specific, fixed starting point at the same corner of the corridor between the mirrored chamber and the containment box. Each mouse was allowed to move freely around the corridor during an empirically validated 5 min evaluation period. Latency to enter, number of entries and total time (duration) spent inside the mirrored compartment were scored by an observer approximately 1 m from the apparatus. The criterion for entry into the mirrored chamber was all four feet being placed on the mirrored floor of chamber. Between assessment of individual animals, the apparatus was washed thoroughly with tap water to reduce potential cues left by the previous occupant. Fifteen mice were evaluated for each dose of S-21007 and vehicle. Latency values were expressed as $\bar{x} \pm s$. Significance of the overall dosage-dependent behavioral changes induced by the drug was assessed by ANOVA.

Plus-maze behavior The plus-maze apparatus was made of Plexiglas and consisted of two open arms 30.5 cm long with a 5-cm wide runway and two enclosed arms 30.5 cm long with a 5-cm wide runway enclosed by clear Plexiglas walls 15 cm high. The arms extended from a central platform, and the runways of both arms were made of black Plexiglas. The apparatus was similar to that described by Lister (1987). Behavioral evaluations were carried out in a quiet room, with a fluorescent lighting (constant lighting of 200 lux). Animals were brought in the room 30 min prior to experimentation. During the test period, the mouse was placed in the center of the plus-maze facing an open arm. During the five-minute test, the delay of entry as well as the number of entries and time spent in each of the two arms were scored by

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direct observation. A mouse was taken to have entered an arm when all four legs were on the arm (outside the start position). All results are expressed as $\bar{x} \pm s$. Ten mice were evaluated per data point. Significance of the overall dosage-dependent behavioral changes was assessed by ANOVA.

RESULTS

Locomotor activity over a 90-min period

Repeated measures ANOVA indicated a reliable main effect of drug treatment ($F_{45,477} = 2.15$, $P < 0.01$), only due to an increase in the number of transitions in the first 9 min at the dose of 1 $\mu g \cdot kg^{-1}$ ($P < 0.01$ *vs* controls) (Fig 1).

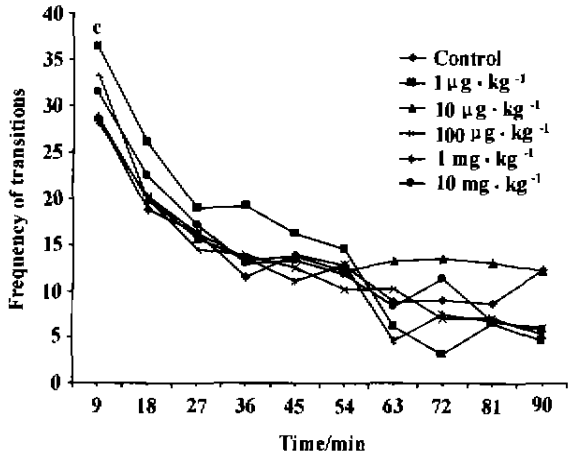

Fig 1. Effect of ip S-21007 on locomotor activity (number of transitions between two boxes) in mice. Frequency of observations for each 9-min time period post-injection. $\bar{x} \pm s$. $^{c}P < 0.01$.

Exploratory responses ANOVA failed to reveal any reliable effects of S-21007 on the number of units entered ($F_{4,44} = 1.32$), the rearing behavior ($F_{4,44} = 0.52$) or the novelty preference ($F_{4,44} = 1.46$) (Fig 2). S-21007 did not modify the exploratory behavior.

Acute injection of S-21007 in the light/dark choice paradigm

Intraperitoneal administrations: ANOVA indicated a reliable treatment effect for both time spent in the lit box ($F_{5,180} = 4.59$, $P < 0.01$) and number of transitions between the two boxes ($F_{5,180} = 4.01$, $P < 0.01$). Bonferroni's *a posteriori* comparisons

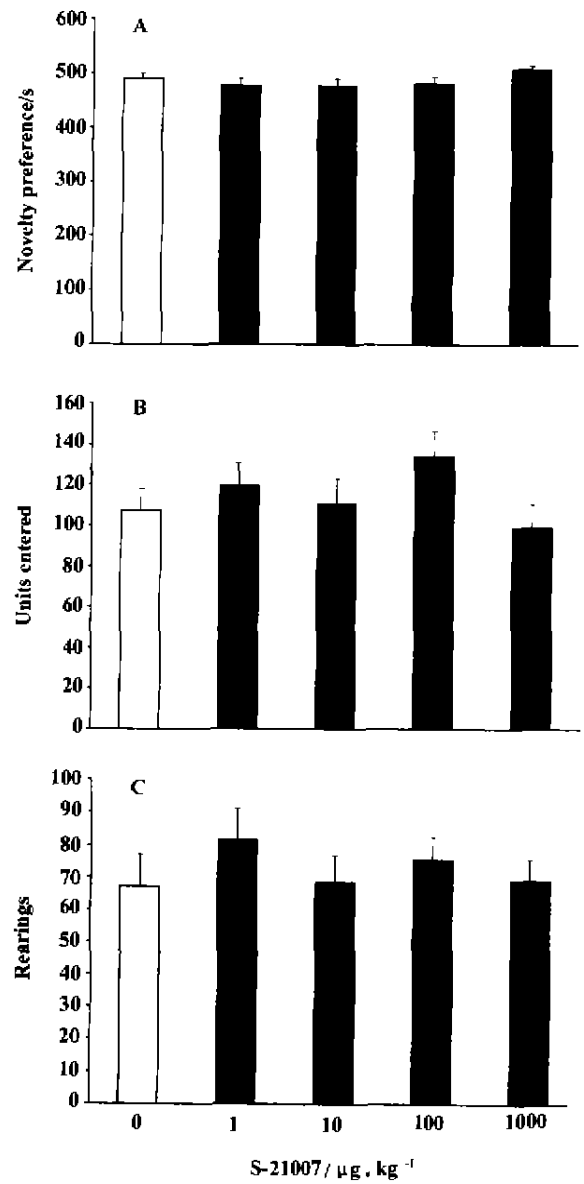

Fig 2. Free-exploration test: A) time spent by mice in the novel compartment (novelty preference); B) locomotion (number of units entered) and C) number of rearings exhibited by animals given a 10-min test 30 min after ip administration of S-21007. $\bar{x} \pm s$.

between S-21007 treated groups and the saline control group revealed an increase in the time spent in the illuminated box and a raise in the occurrence of transitions at all doses tested (10 ng – 100 $\mu g \cdot kg^{-1}$) (Fig 3).

Oral administrations: Comparisons (ANOVA) of S-21007 treatment and saline-treated control group indicated a reliable effect of drug treatment for both

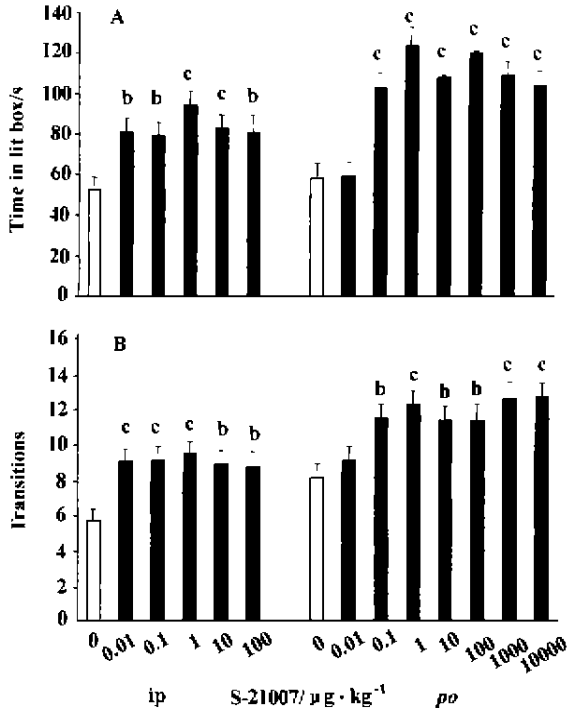

Fig 3. Acute treatment in the light/dark choice procedure. A) Time spent by animals in the illuminated area and B) number of transitions between the lit area and dark box in mice given a 5-min test 30 min following ip or *po* administrations of S-21007. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$.

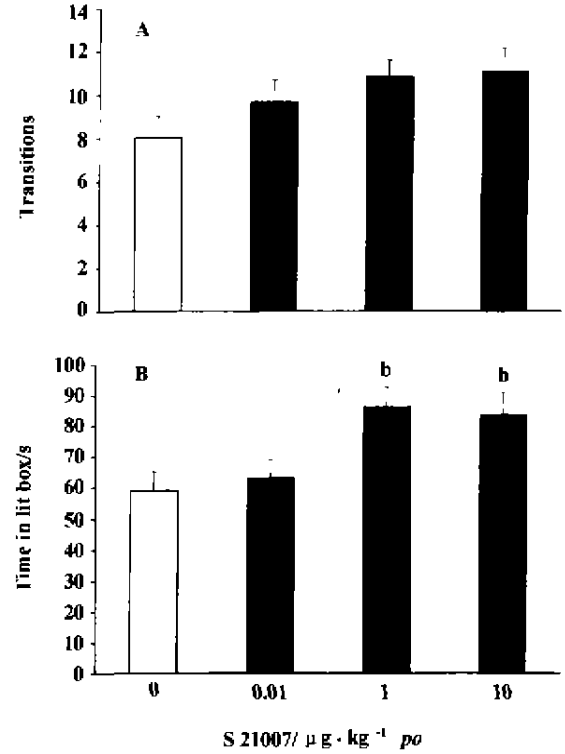

Fig 4. Chronic treatment in the light/dark choice procedure: mice received *po* saline (control group) or a given dose of S-21007 once for 14 d. At d 15 and 30 min before the test, control group received a saline injection and drug treatment groups a dose of S-21007. A) Time spent by mice in the illuminated area and B) number of transitions between the two boxes. $\bar{x} \pm s$. $^bP < 0.05$.

time spent in the lit box ($F_{7,242} = 10.20$, $P < 0.01$) and number of transitions between the two boxes ($F_{7,242} = 4.33$, $P < 0.01$). Bonferroni post-hoc comparisons between S-21007 treated groups and the saline control group confirmed an increase in the time spent in the illuminated box and a raise in the occurrence of transitions from 100 ng·kg^{-1} to 10 mg·kg^{-1} (Fig 3).

Chronic treatment of S-21007 in the light/dark choice procedure ANOVA revealed a reliable main effect of dose for the time spent by mice in the illuminated box ($F_{3,56} = 4.74$, $P < 0.01$) but not for the number of transitions ($F_{3,56} = 2.09$). Subsequent post-hoc analysis with the Bonferroni's *t*-test indicated a significant increase in the time spent in the lit box at 1 and 10 μg·kg^{-1} (Fig 4).

Mirrored chamber aversion test Individual doses from 1 to 100 μg·kg^{-1} significantly increased total time compared to vehicle treated controls and significantly reduced latency to enter the mirrored chamber (Fig 5). The overall dosage-dependent action of S-21007 was significant ($P < 0.05$) on reduction in latency to enter in the mirrored chamber but was not significant for the time spent due to high variance. The maximal reduction in latency to enter in the chamber was from a control value of (290 ± 5) to (177 ± 26) s. The total time spent inside the mirrored chamber increased from (2.2 ± 1.0) s in vehicle treated animals to (24 ± 8) s in mice receiving S-21007 10 μg·kg^{-1} ip (Fig 5).

Elevated plus-maze test Significant decreases in latency to enter occurred at both the 10 and 100 μg·kg^{-1} ip doses (Fig 6). The maximal reduction in latency to enter, from (298 ± 2) s in vehicle treated control animals to (104 ± 31) s occurred in mice receiving the dose of 100 μg·kg^{-1}. The total time on the open arms of the elevated plus maze also showed a

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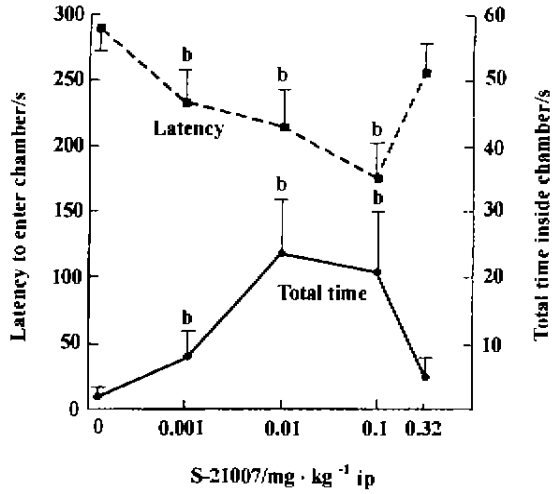

Fig 5. Mirrored chamber aversion test: activity of S-21007 after ip administration. $\bar{x} \pm s$. $^bP < 0.05$.

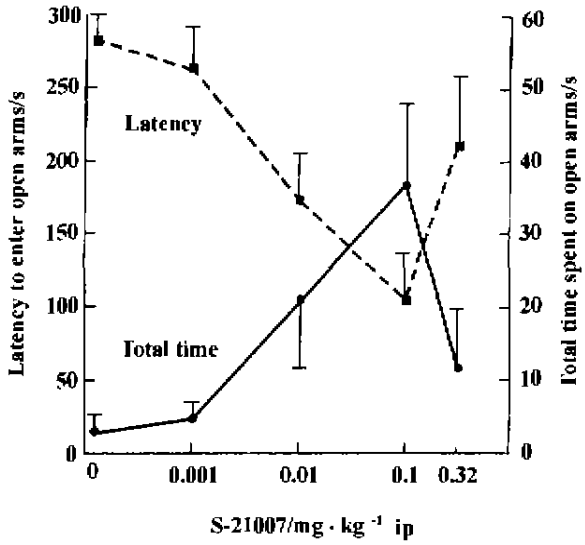

Fig 6. Elevated plus-maze test: activity of S-21007 after ip administration. $\bar{x} \pm s$. $^bP < 0.05$.

statistically significant increase. Maximal increases in time spent in the open arm (37 ± 11) s *vs* (2 ± 2) s occurred at 100 $\mu g \cdot kg^{-1}$ ip. The overall dosage effect on reduction of latency to enter the open arms of the plus-maze was highly significant ($P = 0.0002$).

DISCUSSION

S-21007 is a potent and selective 5-HT_3 compound[3] which has been characterized as a partial agonist in different *in vitro* and *in vivo* models[4]. In this study, S-21007 presented anxiolytic-like activities in three models well characterized for revealing anxiolytic agents: the light/dark box test, the elevated plus-maze test[6] and the mirrored chamber test[5]. This effect appeared after ip and oral treatments, at very low doses, for example 10 $ng \cdot kg^{-1}$ in the light/dark box procedure and 10 $\mu g \cdot kg^{-1}$ in the elevated plus-maze and the mirrored chamber aversion tests and was maintained after chronic treatment. These low effective doses are a property common to the 5-HT_3 antagonists active in this model[1,6]. This anxiolytic-like activity was devoid of sedative effect, unlike benzodiazepines in the locomotor activity test. The weak increased transitory of locomotor activity observed at the lowest dose, during the first 9 min, did not interfer with the different tests which were all performed 30 min after treatment. Lastly, S-21007 did not affect or disorganize the exploratory behavior of mice.

S-21007 was much more active in the light/dark box test and on wider range of doses than in the plus-maze or mirrored chamber tests. This difference in potency could be due to the difference (1) in the test and (2) in the mice strains studied. The light/dark box test appears as a sensitive test for 5-HT_3 antagonist since most of them have anxiolytic properties in this test when perform in mice[1,6]. This was not the case for the elevated plus-maze for which the results for 5-HT_3 antagonists are very contradictory even for the same compound[1,6,7]. Less data have been obtained in the mirrored chamber but diazepam and 5-HT_{1A} agonists were active in this model[5]. In the same experiment the 5-HT_3 antagonist, MDL 72222 exhibited anxiolytic-like activity at higher doses than S-21007 (data not shown). Generally, it seems that the type of aversive stimuli used in the models of anxiety are important to demonstrate an anxiolytic activity for 5-HT_3 ligands. For example, in the social interaction test where it is possible to associate two different parameters, light (low or high) and familiar or unfamiliar conditions, most of 5-HT_3 antagonists are active in this model only under the most aversive conditions (high light and unfamiliar)[1,7].

The weakest efficacy observed in the plus-maze and mirrored chamber test could also be due to the different mice strains studied in this model. Heterogeneity of 5-HT_3 receptors within different mice

strains has been reported[8] and S-21007 may have different affinities for the 5-HT_3 receptors of the two mice strains.

S-21007 has been described so far as a specific 5-HT_3 compound active in anxiolytic tests where 5-HT_3 antagonists were more and less active. But S-21007 has been characterized in pharmacological models as a partial agonist[4]. 5-HT_3 receptors have been localized mainly on nerve fibers and terminals in different central nervous structures such as the hippocampus and amygdala[9], which are involved directly or indirectly in the mechanisms of anxiety and by the way could regulate the release of transmitters. The two major neurotransmitters involved in the mechanisms of anxiety are 5-HT and cholecystokinin (CCK). Both of them have their release partly controlled by 5-HT_3 receptors. The selective 5-HT_3 agonist 2-methyl-5-hydroxytryptamine (2-Me-5-HT) directly infused into the rat hippocampus increased 5-HT levels[10] and the selective 5-HT_3 antagonists ondansetron and tropitron infused in the rat cortex antagonized the depolarization-evoked CCK release[11]. Costall *et al*[12] showed using the light/dark box procedure that microinjection of 2-Me-5-HT into the amygdala increased anxiety whereas the 5-HT_3 antagonist ICS 205930 in the same conditions induced anxiolytic response. These observations are in agreement with that stimulation of 5-HT_3 receptor by agonist will increase 5-HT concentrations and will thus increase the level of anxiety (reactivity).

The first hypothesis to explain the anxiolytic properties demonstrated in this study for the partial 5-HT_3 agonist S-21007 could be a desensitization of the 5-HT_3 receptors, giving by this way the same profile than 5-HT_3 antagonists. This rapid desensitization of the 5-HT_3 receptors was also reported by Blier et Bouchard[13] using 2-Me-5-HT application on hypothalamus, hippocampal, and frontal cortex slices of guinea pig and the stimulated release of [^{3}H] 5-HT. The first application of 2-Me-5-HT elicited a release of 5-HT, whereas a second application had no effect. Nevertheless, they did not observe the same agonistic effects with the potent selective 5-HT_3 agonist phenylbiguanide which behaved like an antagonist against 2-Me-5-HT. The authors mentioned the results obtained *in vitro* by Yakel et Jackson[14]: they reported a rapid desensitization which occurred within a few seconds. S-21007 by its higher affinity than phenylbiguanide and 2-Me-5-HT for the 5-HT_3 receptors[4] could perhaps induce a more rapid desensitization. The weak increase in locomotor activity observed for S-21007 during the first minutes is probably due to this short agonistic effect.

The second hypothesis is founded on the definition of a partial agonist. According to Stephenson[15], a partial agonist is a drug that produces submaximal responses compared to the endogenous ligand and competitively blocks the effects of the endogenous ligand or agonists of higher intrinsic efficacies. Indeed S-21007 had only a weak (locomotor activity) or no effect (exploratory test) in non adversive tests but had anxiolytic-like activity in adversive situation, ie, when 5-HT levels are increased.

Biochemical studies following the 5-HT levels in different structures such as hippocampus or amygdala by microdialysis in anxiogenic situations will allow to understand the mechanisms of actions of S-21007. Preliminary results show that in rat after chronic treatment (10 d) S-21007 I mg · kg^{-1} in normal conditions decreases 5-HT content *ex vivo* in the hippocampus (76 % ± 6 % of control values).

In conclusion, S-21007, a selective 5-HT_3 partial agonist, exhibited anxiolytic-like properties in different animal models of anxiety. The mechanism of action is not yet established and could pass through desensitization of 5-HT_3 receptors or antagonist effect on different 5-HT_3 receptor subtypes.

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S-21007，强效 5-羟色胺 3 受体部份激动剂，对小鼠焦虑的作用

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Effects of modafinil and amphetamine on sleep-wake cycle after sleep deprivation in cats

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ABSTRACT

AIM: The effects of modafinil and amphetamine on sleep-wake cycle and cortical power spectrum were assessed in the cats before and after sleep deprivation. **METHODS**: The sleep deprivation in the cats was used with the water tank technique. Cats were administrated with modafinil (5 mg · kg^{-1} *po*) or amphetamine (1 mg · kg^{-1}) before and after sleep deprivation. **RESULTS**: The waking effect of 8 - 10 h induced by modafinil before and after sleep deprivation was similar and was not followed by an increase in sleep rebound. On the contrary, the arousal effect about 8 h evoked by amphetamine after sleep deprivation was less lasting than that of 10 - 12 h observed in normal conditions and followed by an amplified rebound in both deep slow wave sleep and paradoxical sleep. **CONCLUSION**: These results suggest the efficiency of modafinil against somnolence and hypersomnia without increasing subsequent sleep.

INTRODUCTION

The sleep deprivation in the cat, as in the other species including man, induces a subsequent increase in cortical slow activity (δ and Θ waves, 0.5 - 8 Hz)[1,2], and the time spent in slow wave sleep (SWS) and paradoxical sleep (PS)[3,4]. During this recovery period, the increased need for PS and SWS constitutes a experimental model of somnolence to study the indications of waking agents in the treatment of hypersomnia and narcolepsia in man.

Modafinil is a newly-synthesized molecule known for its dose related induction of wakefulness in several species[5,6]. Unlike amphetamine, a well-known catecholamine-releasing agent, it elicits an arousal effect without significant sleep rebound in normal cat[6]. It is unknown, however, if modafinil has the same arousal effect in the animal models of experimental somnolence. The present study was designed to investigate whether modafinil and amphetamine were able to evoke wakefulness in the cat when the sleep pressure increases after its deprivation and how they modified the phenomena of sleep rebound.

MATERIALS AND METHODS

Electrodes implanting Six adult cats (Lyon Animal Center, no distinction of race) of both sexes weighing 2.9 - 4.1 kg were chronically implanted, under pentobarbital anesthesia (25 mg · kg^{-1} iv), with electrodes for polygraphic recordings of neocortical and hippocampal electroencephalogram (EEG), ponto-geniculo-occipital (PGO) activity, electromyogram (EMG) and electrooculogram (EOG). In addition, a thermistor (10 K3 MCD2, Betatherm, 10 kΩ at 25 ℃, outer diameter of 0.45 mm) was placed in the caudate nucleus to record brain temperature. After a recovery period of ten days, the cats were housed in a sound-attenuated and dimly illuminated cage at 22 - 25 ℃ and fed daily at 6 pm (the day of sleep deprivation at 3 pm). Polygraphic recordings were performed for 4 d to obtain the basic qualitative and quantitative parameters of sleep-wake cycle. The neocortical and

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hippocampal EEG was recorded during the experiences by a signal acquisition system CED (Cambridge Electronic Design) and treated with the program Spike 2.

Sleep deprivation The sleep deprivation was performed by water tank technique[3,4] during 18 h (from 4 pm to 10 am). After being removed from the water tank, cats were let to groom during 15 min before receiving one of the following oral administrations: placebo, modafinil (5 $mg \cdot kg^{-1}$) and amphetamine (1 $mg \cdot kg^{-1}$). The concert method administered an empty capsule as placebo, an including modafinil or amphetamine capsule respectively into cat oral cavity and followed some water. Each cat must be submitted to a deprivation before each of the three administrations in a random manner. Subsequent recordings were made for 72 h. There was an interval of 7 d between administrations.

Polygraphic recording In order to compare the waking effect of modafinil and amphetamine in the normal conditions and after sleep deprivation, some cats received the same drugs one week before the sleep deprivation. The induced waking-duration was defined, before or after sleep deprivation, by the time from the onset of first waking episode equal or superior to 20 min to the appearance of the first deep slow wave sleep (S2) episode equal or superior to 2 min. The latency to S2 after placebo was made from the moment of administration because a waking episode of 20 min was usually absent. Polygraphic recordings were scored min by min according to previously described criteria for wakefulness (W), light slow wave sleep (S1), deep slow wave sleep (S2) and paradoxical sleep (PS).

Statistical analysis The data were presented as $\bar{x} \pm s$ and evaluated by t-test and ANOVA.

RESULTS

Sleep deprivation by water tank technique during 18 h in the cat induced significantly a decrease in the latency to S2 [(18 ± 8) *vs* (55 ± 12) min in the normal conditions] and an increase in S2 and PS during recovery phase. Moreover, power spectrum of neocortical slow activity (0.5 to 4 Hz) increased during SWS (Fig 1). These sleep rebounds phenomena were mostly observed during the first 12 – 16 h following sleep deprivation.

In agreement with the previous results[6], oral application of modafinil at a dose of 5 $mg \cdot kg^{-1}$ induced a waking effect of 8 – 10 h in the normal conditions, whereas that of amphetamine at a dose of 1 $mg \cdot kg^{-1}$ evoked a waking period of 10 – 12 h.

After sleep deprivation, both modafinil and amphetamine were capable of inducing a long lasting waking state (Fig 1). Throughout this period, the cats were attentive, cortical slow activity was suppressed (Fig 1) and the spectrum analysis of hippocampal EEG showed a persistence of Θ waves (not shown). The duration of the waking effect of modafinil after sleep deprivation was similar to that observed in normal condition. The waking duration evoked by amphetamine after sleep deprivation, however, shortened as compared with that observed in the normal conditions (Tab 1).

Tab 1. The duration (time in min) of waking effect of placebo, modafinil (5 $mg \cdot kg^{-1}$, *po*) and amphetamine (1 $mg \cdot kg^{-1}$, *po*) after sleep deprivation (SD). $n = 6$. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* without SD.

	Placebo	Modafinil	Amphetamine
Without SD	45 ± 5	506 ± 14	682 ± 15
After SD	23 ± 4[b]	511 ± 14[a]	475 ± 14[c]

Following the waking effect of modafinil, the appearance for both SWS and PS was similar to that observed with placebo during the same period. The power spectrum and frequency of slow activity of neocortical EEG were not obviously modified. The evolution of each sleep-wake stage subsequent to arousal was approximately parallel to that obtained with placebo, so that a significant difference for cumulative values of W, S1, S2, and PS between the two curves persists at the end of 48 h recordings ($P < 0.01$) (Fig 2).

On the contrary, after the arousal effect evoked by amphetamine, the rebound in both S2 and PS occurred more intensively than that observed with placebo. Hence, the cures of each sleep-wake stage following the amphetamine-induced wakefulness approached gradually to that obtained with placebo. The cumulative values of each stage obtained with amphetamine did not show

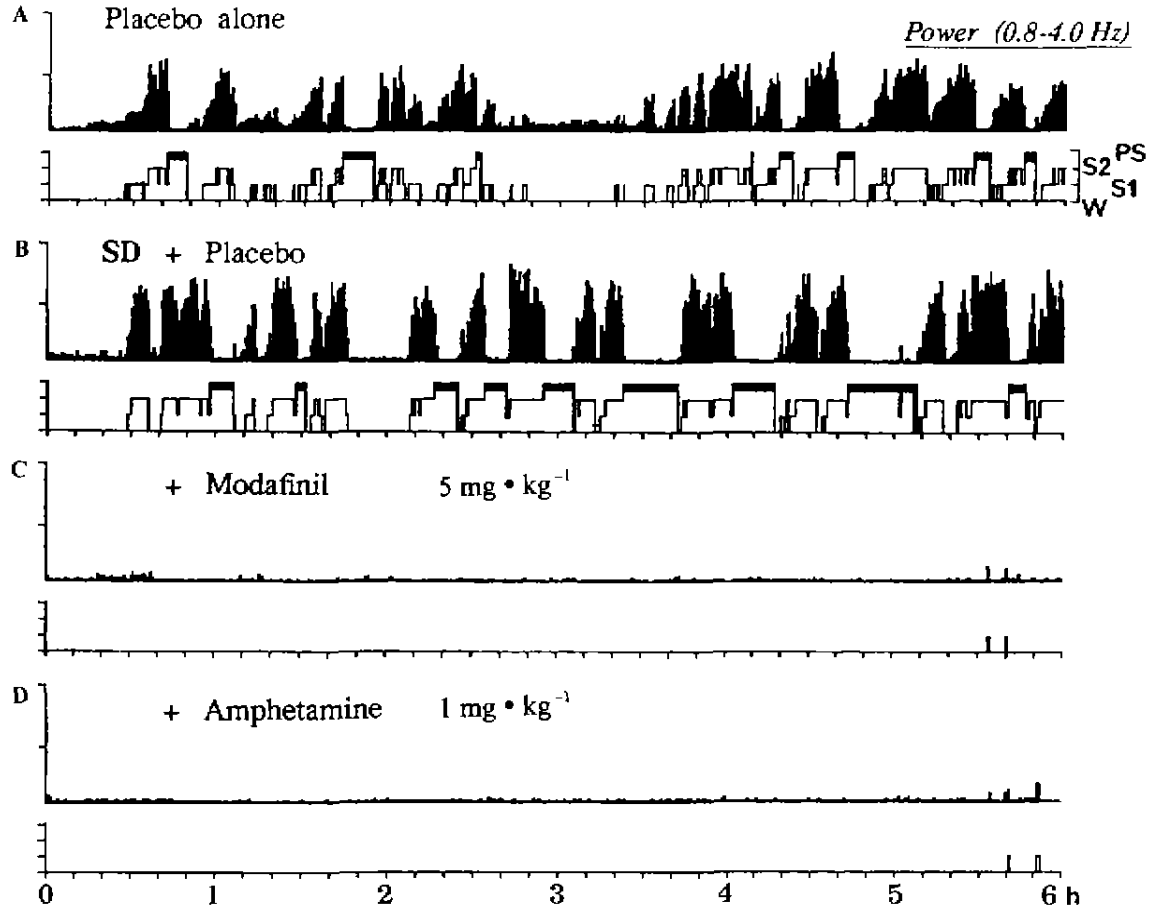

Fig 1. Representative 6-h hypograms and cortical power density (0.8 – 4.0 Hz) showed a control recording following administration of placebo alone and the effects on sleep and wakefulness following oral applications of placebo, modafinil or amphetamine after sleep deprivation. Note, after sleep deprivation, a sleep rebound following application of placebo and the waking effects of modafinil and amphetamine. SD, sleep deprivation. Ordinate: Power, power spectrum of neocortical EEG; W, wakefulness; S1, light slow wave sleep; S2, deep slow wave sleep; PS, paradoxical sleep. Abscissa: time in hour.

statistical difference to that obtained with placebo at end of 48-h recordings ($P > 0.05$ for all). They do differ significantly from that obtained with modafinil ($P < 0.01$) (Fig 2).

An analysis for 20 h during recovery phase (which is indicated on the abscissa of each stage in Fig 2) further indicated less time spend in W while more time spend in PS and S2 after administration of amphetamine than after administration of modafinil and placebo ($P < 0.01$, upper windows on each part of Fig 2). However, S1 obtained with three treatments did not show significant difference during this period.

DISCUSSION

In the present study, the cats deprived of sleep during 18 h showed subsequently a significant decrease in the latency to S2 and an increase in the cortical slow activity as well as the duration of S2 and PS, suggesting an increased sleep pressure. These results confirm the previous studies in different species[1-4,7] and demonstrate the validity of the method used as a reversible model of experimental somnolence for studying the indications of waking molecules.

Even though the sleep deprivation rendered the need for sleep imperative, modafinil was able to induce a waking effect identical to that observed in the normal conditions and therefore extended the duration of sleep deprivation. In spite of this, it did not amplify sleep rebound. In contrast, the duration of the waking effect of amphetamine decreased after sleep deprivation and