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Short communication

Antidepressant-like effects of alnespirone (S 20499) in the learned helplessness test in rats

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Abstract

The effects of the new chroman derivative, alnespirone (S 20499), which is a selective 5-HT_{1A} receptor agonist, were investigated in an animal model of depression, the learned helplessness test. Rats previously submitted to a session of 60 inescapable electric foot shocks (learned helpless controls) exhibited a deficit in escape performance in three subsequent shuttle-box sessions. Alnespirone was administered twice daily via the oral route (2.5, 5, 10, 20 mg kg^{-1} day^{-1}). It was shown to protect against the elevation in escape failures caused by exposure to the uncontrollable aversive situation at 5 and 10 mg kg^{-1} day^{-1} p.o. (13 ± 2 and 10 ± 3 escape failures, respectively, vs. 9 ± 2 escape failures in control rats). In addition, alnespirone had a tendency to elevate the number of intertrial crossings during the resting periods, depending on the dose and day on which the avoidance task was performed (15 ± 2 intertrial crossings at the dose of 5 mg kg^{-1} day^{-1}, vs. 5 ± 2 intertrial crossings for the helpless control rats, on the second day). In comparison, imipramine (64 mg kg^{-1} day^{-1} p.o.) provided marked protection on all three days of the avoidance task and tended to increase the number of intertrial crossings during the resting periods on the second and the third days. It is concluded that alnespirone exerts antidepressant-like properties in the learned helplessness test in rats, in a manner similar to 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin), buspirone and ipsapirone, other 5-HT_{1A} receptor agonists. © 1998 Elsevier Science B.V.

Keywords: Alnespirone; Learned helplessness paradigm; Antidepressant effect; 5-HT_{1A} receptor

1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) has long been implicated in the biology of depression as well as in the mechanism of action of antidepressant agents (Stahl, 1994). This relationship between 5-HT and depression was reinforced by the discovery of various 5-HT receptor subtypes. The concept of multiple types of 5-HT receptors has led to the suggestion that drugs with a selective action at certain brain 5-HT receptors may have particular psychotropic properties in affective disorders.

5-HT_{1A} receptors are located both presynaptically, as somatodendritic (auto) receptors on the cell bodies in the dorsal and median raphe nuclei, and postsynaptically, predominantly in the hippocampus and the lateral septum (Palacios et al., 1987). It has been suggested that presynaptic receptors are predominantly involved in the anxiolytic effects of 5-HT_{1A} receptor ligands (Higgins et al., 1988) and that postsynaptic receptors are mainly involved in the antidepressant effects of these ligands (Martin et al., 1990). However, recent evidence suggests that presynaptic receptors may also be involved, at least partially, in the antidepressant effects (Redrobe et al., 1996).

Evidence implicating 5-HT_{1A} receptors in the pathophysiology of depression and mechanism of action of antidepressant drugs has been obtained from various biochemical, behavioural and electrophysiological studies in animals (Newman et al., 1993). Some 5-HT_{1A} receptor agonists, such as buspirone and ipsapirone, have been shown to possess antidepressant properties in several animal models as well as in clinical studies (Robinson et al., 1989). 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin), the reference 5-HT_{1A} receptor full agonist, has the same effects in several rat models of depression (De Vry and Schreiber, 1993).

Alnespirone is a new selective and potent 5-HT_{1A} receptor agonist, with both in vivo and in vitro pre- and postsynaptic agonist properties (Kidd et al., 1993). Radioligand binding studies have shown this compound to have a

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high affinity for 5-HT_{1A} receptors ($K_{0.5} = 0.2$ nM). Like 8-OH-DPAT, alnespirone has been shown, using autoradiography, to bind specifically and strongly to the dorsal raphe nucleus, the hippocampus and the lateral septum (Lanfumey et al., 1993). Unlike buspirone, which is a dopamine D_2 receptor antagonist, alnespirone is devoid of direct dopaminergic D_2 activity (Chouvet et al., 1994) and does not release the metabolite, 1-pyrimidyl-piperazine, which is a potent α_2-adrenoceptor antagonist and can impair the antidepressant effects of the parent drug (Martin, 1991). Alnespirone shows marked anxiolytic activity in various models with pigeons, rats and mice at low doses and via different routes of administration (Porsolt et al., 1992; Griebel et al., 1992; File and Andrews, 1994; Barrett et al., 1994; Curle et al., 1994; Charrier et al., 1994). The consistency and reproducibility of the anxiolytic activity of alnespirone sets it apart from other 5-HT_{1A} receptor agonists. Furthermore, no withdrawal syndrome was found in rats following discontinuation of chronic treatment (Goudie et al., 1994).

These data prompted us to investigate the effects of alnespirone in the learned helplessness model of depression, since it has been shown that pharmacological agents used for the treatment of human depression are active in this model (Sherman et al., 1982). The model involves exposure of the animals to inescapable shock treatment and observation of the subsequent retardation in the acquisition of tasks using appetitive or aversive reinforcers. The reversal of learned helplessness (i.e., restoration of normal responding) can be achieved by chronic administration of reuptake inhibitor agents or 5-HT_{1A} receptor agonists (Giral et al., 1988).

2. Materials and methods

2.1. Animals

The experiments were carried out using male Wistar rats (Centre d'élevage R. Janvier, France) weighing 180–220 g at the beginning of the experiments. The animals were housed in groups of 10 per cage under standard conditions: room temperature (21 ± 1°C); light/dark cycle (12/12 h); water and food ad libitum.

2.2. Apparatus and experimental procedure

2.2.1. Learned helplessness procedure

The procedure has been described earlier (Sherman et al., 1982). Briefly, it includes two phases.

(1) Helplessness induction (inescapable shock preconditioning): On day 1, rats were isolated in small altuglass boxes (20 cm × 20 cm × 10 cm) covering a stainless-steel grid. A constant-current shocking device was used to deliver 60 scrambled randomized electric foot shocks (0.8 mA) for 15 s every min (i.e., for 1 h). Control rats were placed on the grid in identical chambers for 1 h, without receiving the uncontrollable electric shocks. After this session, animals were replaced in their own cages.

(2) Conditioned avoidance training: To evaluate escape deficits, on day 3, i.e., 48 h after exposure to the electric shocks, the animals were submitted to an avoidance task in a shuttle box. The shuttle box consisted of two equal-sized compartments divided by a stainless steel partition fitted out with an opening (7 cm × 7 cm), and the grid floor consisted of stainless-steel rods spaced 1-cm apart. A photoelectric system ensured that electrification of the grid floor stopped at each crossing of the animal into the other compartment.

The animals were placed singly into the shuttle box and allowed to habituate to the environment for 5 min. Following this free exploration session, 30 stimulus-shock trials were presented for a period of 15 min, i.e., two trials per minute. During the first 3 s of each trial, a light signal was presented, followed by a 3-s electric shock (0.8 mA) applied via the grid floor and then by a 24-s resting period. Shuttle-box sessions were performed on three consecutive days (days 3, 4 and 5).

In this paradigm, animals responded in one of two ways: (1) During the light signal, they crossed into the other compartment, either having partially received (escape) or not having received (avoidance), the electric shock. (2) They received the electric shock, i.e., they failed to escape the aversive situation.

The number of escape failures, referred to as a 'non-crossing response' during stimuli presentation and the number of intertrial crossings observed during the resting period were recorded.

2.3. Drug administration

Drugs were administered orally in half doses twice daily, 1 h before the avoidance task and between 1800 and 1900 h. This treatment was carried out on 5 consecutive days, apart from day 1 when the daily dose was given 6 h after induction of learned helplessness, as a single bolus. Rats were randomized into groups of 10. Alnespirone was administered at daily doses of 2.5, 5, 10 and 20 mg kg^{-1} day^{-1} p.o.; the reference drug imipramine was administered at the daily dose of 64 mg kg^{-1} day^{-1} p.o. The two control groups (with electric shock treatment: helplessness controls, or without electric shock treatment: nonhelplessness controls) were given the solvent. Alnespirone and imipramine were dissolved in isotonic saline. The injection volume was 0.5 ml/100 g bodyweight.

2.4. Statistical analysis

The number of escape failures was recorded on days 3, 4 and 5 (expressed as mean + S.E.M), for each group of

rats with $n = 10$ animals per group. The number of intertrial crossings observed during the resting periods was also recorded. All differences were considered statistically significant when the null hypothesis could be rejected at the risk $\alpha = 0.05$. Data were submitted initially to an analysis of variance (ANOVA) with days as factors for each group. This was followed by between-group comparisons for each shuttle-box session, using a one-way analysis of variance (ANOVA).

3. Results

3.1. Control groups

As previously described (Martin et al., 1986), non learned helpless control animals, i.e., those that were not submitted to the uncontrollable aversive situation on day 1, failed to escape the electric shocks during the first day of the avoidance task (9 ± 2 escape failures over the 30 imposed stimulus-shock trials) (Fig. 1). The escape failures remained steady on the second and the third days (9 ± 3 and 9 ± 2, respectively). There were several intertrial crossings during the resting periods of these three days (18 ± 6, 18 ± 5 and 11 ± 3, respectively) (Fig. 2). In contrast, the learned helpless control animals, i.e., those that received the uncontrollable electric shock on day 1, showed a much higher number of escape failures on the first day of the avoidance task (18 ± 2; $P = 0.005$). The escape failures increased slightly on days 2 and 3 (21 ± 2 and 22 ± 2, respectively). Few intertrial crossings were observed (7 ± 1, 5 ± 2 and 6 ± 2, respectively).

The increase in the number of escape failures in the animals that received the inescapable electric shocks was indicative of a behavioural deficiency caused by exposure to an uncontrollable aversive situation, which is considered to be related to the emergence of depression.

3.2. Effects of alnespirone

After oral administration, alnespirone reduced the increase in escape failures caused by the inescapable electric shocks delivered on day 1. This effect was significant at 5 mg kg^{-1} day^{-1} on the last two days of the avoidance task (13 ± 2, $P = 0.02$; 11 ± 2, $P = 0.003$ escape failures, respectively), and at 10 mg kg^{-1} day^{-1} on the first and the third days (10 ± 3, $P = 0.03$; 13 ± 3, $P = 0.02$, respectively). The lowest (2.5 mg kg^{-1} day^{-1}) and highest (20 mg kg^{-1} day^{-1}) doses were inactive (Fig. 1). In addition, alnespirone tended to elevate the number of intertrial crossings during the resting periods, depending on the dose and on the day of avoidance task, and the effect became significant at 5 mg kg^{-1} day^{-1} on the second day (15 ± 2, $P = 0.01$). Values increased towards those of the non helpless control group, but did not exceed them.

In comparison, the reference compound, imipramine, exerted a marked protection on all three days of the avoidance task (9 ± 2, 5 ± 2, 8 ± 3, $P < 0.0001$ escape failures, respectively), and it tented to increase the number of intertrial crossings during the resting periods, on the

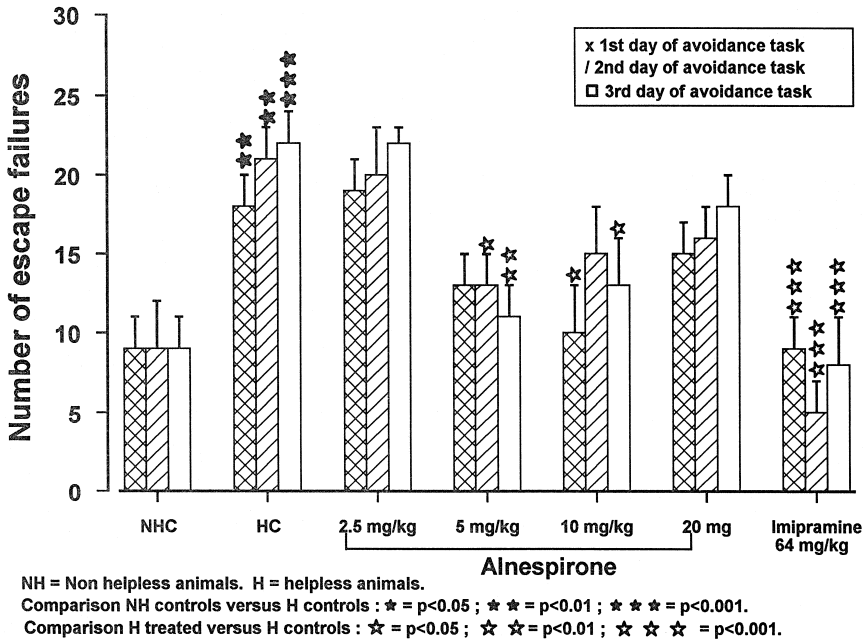

Fig. 1. Number of escape failures observed during three days of an avoidance task (shuttle box) in the learned helplessness test in rats. Protection displayed by alnespirone (p.o.). Comparison with imipramine (p.o.). M + S.E.M. with 10 to 12 animals per group.

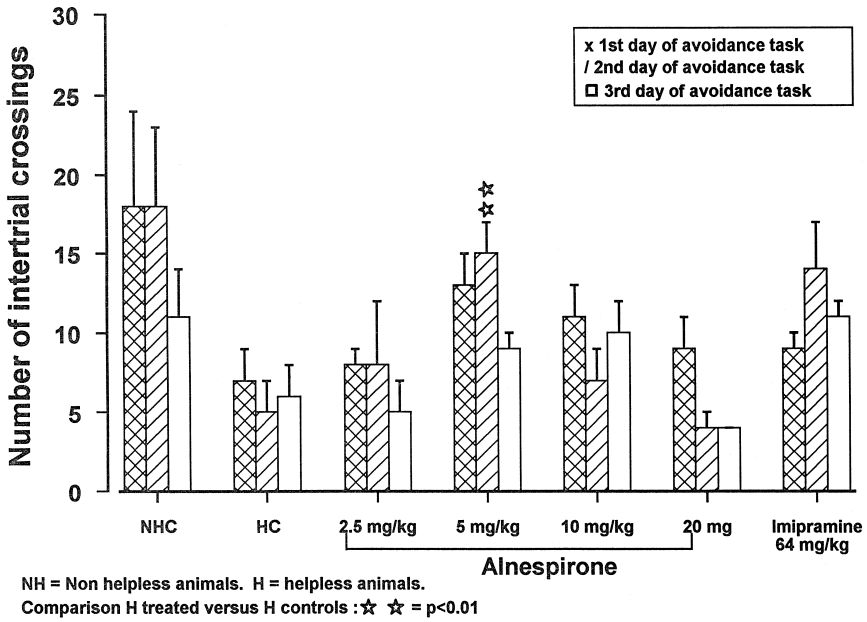

Fig. 2. Number of intertrial crossings observed during three days of an avoidance task (shuttle box) in the learned helplessness test in rats. Protection displayed by alnespirone (p.o.). Comparison with imipramine (p.o.). M + S.E.M. with 10 to 12 animals per group.

second and the third days (14 ± 3, $P = 0.06$; 11 ± 1, $P = 0.07$, respectively).

4. Discussion

This study shows that alnespirone, a selective pre- and postsynaptic 5-HT_{1A} receptor agonist, administered twice daily (5 and 10 mg kg^{-1} day^{-1} p.o.) after a session of inescapable foot shocks, reversed the escape deficits typically observed in subsequent shuttle-box training. This effect is characteristic of antidepressant agents administered according to a comparable procedure (Sherman et al., 1982; Martin et al., 1986). In addition, Graeff et al. (1989) found that ipsapirone, a partial 5-HT_{1A} receptor agonist, reversed the learning deficit caused by uncontrollable shocks in the learned helplessness paradigm. Similar findings have been reported by Giral et al. (1988) and Martin et al. (1990) for several 5-HT_{1A} receptor agonists, such as buspirone (0.5 and 1.0 mg kg^{-1} i.p.) and geprione (0.06, 0.125 mg kg^{-1} i.p.).

The increase in intertrial crossings does not indicate a stimulant effect (Detke et al., 1995), but simply reflects the antidepressant effect, as a behaviourally distinct process.

Interpretation of the behavioural effects of receptor-selective compounds is complicated as it is not always easy to separate the contributions of the different sites to the overall effect: for example, the contributions of pre- and/or post-synaptic receptors. In the learned helplessness paradigm, 8-OH-DPAT and busprione microinjected into the anterior raphe nuclei (where presynaptic receptors are predominant) did not reverse helpless behaviour. In contrast, both of these 5-HT_{1A} receptor agonists micro-injected into the septum (where postsynaptic receptors are predominant) dose dependently reversed helpless behaviour (Martin et al., 1990; Schreiber and De Vry, 1990). In addition, in rats in which the ascending 5-HT neurons had been destroyed by administration of 5,7-dihydroxytryptamine (5,7-DHT) into the raphe nuclei, the ability of systemically administered 8-OH-DPAT and systemically administered buspirone, to reduce helpless behaviour was still observed (Martin et al., 1991).

The major metabolite of most 5-HT_{1A} receptor agonists, apart from 8-OH-DPAT, is 1-(2-pyrimidyl)-piperazine, which is rapidly and extensively formed in rats and tends to accumulate in the brain (Caccia et al., 1986). 1-(2-pyrimidyl)-piperazine has been found to exhibit no antidepressant-like properties in the rat forced swimming test (Martin, 1991), to increase locus coeruleus activity via α_2 receptor blockade (Engberg, 1989) and to produce anxiety-like behaviour and behavioural depression (Weiss et al., 1985). The formation of 1-(2-pyrimidyl)-piperazine may alter some effects of 5-HT_{1A} receptor agonists in vivo. Indeed, 1-(2-pyrimidyl)-piperazine antagonises the effects of 8-OH-DPAT, buspirone, gepirone and ipsapirone (Martin, 1991). Owing to its particular chemical structure, alnespirone does not give rise to 1-(2-pyrimidyl)-piperazine as a metabolite. Therefore, the apparent inactivity of alnespirone at the dose of 20 mg k^{-1} day^{-1} cannot be due to 1-(2-pyrimidyl)-piperazine. This inversed U-shaped curve was already observed in tests with high doses and remains unexplained. The dose of 20 mg kg^{-1} day^{-1} is the minimal dose used in toxicity studies. At this dose, the amount of drug the animal is exposed to, in terms of area under the curve (AUC) is ten times higher than that which

humans are exposed to after receiving the maximal tolerated dose, i.e., 20 mg.

In conclusion, administered orally, alnespirone displays a capacity to reverse the behavioural deficiency caused by exposure to an uncontrollable aversive situation, in the learned helplessness test in rats. Protection was observed at 5 and 10 mg kg^{-1} day^{-1}, i.e., at doses not much higher than those shown to produce anxiolytic effects in rats. These findings suggest that alnespirone may be useful as a potential antidepressant agent as, indeed, may other compounds that are active at the 5-HT_{1A} receptor.

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