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Alnespirone (S 20499), an Agonist of 5-HT_{1A} Receptors, and Imipramine Have Similar Activity in a Chronic Mild Stress Model of Depression

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MUÑOZ, C. AND M. PAPP. *Alnespirone (S 20499), an agonist of 5-HT_{1A} receptors, and imipramine have similar activity in a chronic mild stress model of depression*. PHARMACOL BIOCHEM BEHAV **63**(4) 647–653, 1999.—A chronic mild stress (CMS) model of depression was used to study an antidepressant-like activity of alnespirone (S 20499), a selective agonist of 5-HT_{1A} receptors. In this model, a substantial decrease in consumption of a palatable sucrose solution over time is observed in rats subjected to a variety of mild stressors. This effect can be reversed by chronic administration of various classes of antidepressant drugs. Chronic (5 weeks) treatment with alnespirone, in a dose range between 1–5 mg/kg/day, gradually and dose dependently reversed the CMS-induced reductions in sucrose consumption without any significant effects in the non-stressed control animals. The onset of action of the most active doses (2.5 and 5 mg/kg/day) and the overall efficacy of alnespirone in the CMS model were comparable to those observed following similar administration of imipramine (10 mg/kg/day). At the lower (0.5 mg/kg/day) and higher (10 and 20 mg/kg/day) doses, alnespirone was ineffective against the CMS-induced deficit in sucrose consumption. These data provide further support for previous suggestions, based on both the clinical observations and animal data, that agonism at 5-HT_{1A} receptors may result in antidepressant action. © 1999 Elsevier Science Inc.

Alnespirone (S 20499) Imipramine 5-HT_{1A} receptors Animal model Chronic stress Antidepressant action Rat

THERE is evidence that 5-HT_{1A} receptor agonists, such as the members of the azapirone family buspirone, ipsapirone, and gepirone, are active in the treatment of anxiety. In addition, clinical and preclinical studies suggest that this class of compounds may also have antidepressant properties [see (8) for review]. 5-HT_{1A} receptors are located both presynaptically (somatodendritic or autoreceptors on the cell bodies in the dorsal and median raphe nuclei) and postsynaptically (mainly in the lateral septum and hippocampus) (25). Although it has been suggested that the anxiolytic properties are predominantly mediated by the presynaptic receptors and the antidepressant properties by the postsynaptic receptors, it remains unclear to which extent the pre- and postsynaptic mechanisms are involved in the antidepressant activity [see (7) for review].

Alnespirone (S 20499) is a new azapirone that is a potent and selective pre- and postsynaptic agonist of the 5-HT_{1A} receptors: the compound completely inhibits the firing rate of 5-HT neurones of dorsal raphe nucleus (15), attenuates the 5-HT release in the forebrain (4), and inhibits the forskoline-induced adenylate cyclase activity in rat hippocampus (15). In vivo studies demonstrated that alnespirone induced some postsynaptic agonistic effects such as hypothermia (33) or increases in the plasma levels of ACTH and corticosterone (16), and these effects were observed at doses higher than those required to elicit presynaptic activity. This suggests that while in

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vitro alnespirone is a full pre- and postsynaptic agonist, in vivo the compound behaves as a full presynaptic and partial postsynaptic 5-HT_{1A} receptor agonist. Binding studies with labeled compounds have shown the highest levels of labeling in the hippocampus, followed by the lateral septum, entorhinal cortex, and dorsal raphe (11). Unlike other azapirones, alnespirone is not metabolized to 1-(2-pyrimidinyl)-piperazine, a compound that antagonizes α_2-adrenoceptors (2) and does not show any direct activity at dopaminergic receptors (9).

Alnespirone has shown anxiolytic activity after single or repeated administration in several animal models (1,6,12,14,30), and antidepressant activity after repeated administration in the learned-helplessness test (17), unavoidable light-stimulus procedure (21), and in the model of bulbectomized rat (20). Furthermore, microdialysis studies have shown that after chronic administration, alnespirone induces desensitization as well as downregulation of the somatodendritic 5-HT_{1A} receptors in the dorsal raphe (5). Such desensitization caused by chronic treatment of direct or indirect 5-HT_{1A} receptor agonists is thought to play an important role in the mechanism of action of antidepressant drugs [see (3) for review]. These data indicate that alnespirone may be an useful agent in the treatment of depression.

Therefore, the aim of this study was to further explore the antidepressant properties of alnespirone in a well-validated animal model of depression, the chronic mild stress (CMS). In this model, animals subjected to a variety of mild stressors for a prolonged period of time show—among other behavioral, biochemical, and physiological impairments—a substantial decrease in their responsiveness to rewarding stimuli, which can be monitored by a decrease in the consumption of 1% sucrose solution [see (35) for review]. In a series of previous studies, it was demonstrated that the CMS-induced inhibition of sucrose intakes can be effectively reversed by chronic treatment with antidepressant drugs, including tricyclics, atypical antidepressants, selective serotonin reuptake inhibitors (SSRIs), and monoamine oxidase inhibitors (MAOIs), as well as repeated electroconvulsive shocks (ECS). On the other hand, nonantidepressant drugs, such as neuroleptics, psychostimulants, or opiates have been found ineffective in the CMS paradigm [see (28,35)].

It was found in this study that 5 weeks of treatment with alnespirone, in a dose range between 1–5 mg/kg/day, caused a gradual and dose-dependent increases in the consumption of sucrose solution in rats subjected to the CMS procedure without any significant effects in nonstressed control animals. The onset of action of the most active doses (2.5 and 5 mg/kg/day) and the overall efficacy of alnespirone in the CMS model were comparable to those observed following similar administration of imipramine (10 mg/kg/day).

METHOD

Animals

Male Wistar rats (Gorzkowska, Warsaw) were brought into the laboratory 2 months before the start of the experiment. Except as described below (see Stress Procedure), the animals were singly housed with food and water freely available, and were maintained on a 12 L:12 D cycle (lights on at 0800 h) at a temperature of 22 ± 2°C. At the start of the experiments, animal weights ranged between 300–350 g. The study was conducted in compliance with the Animal Protection Bill of August 21, 1997, and has been approved by the Bioethical Committee at the Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland.

Stress Procedure

The animals were first trained to consume a 1% sucrose solution; training consisted of eight 1-h baseline tests (twice weekly) in which sucrose was presented, in the home cage, following 14-h food and water deprivation; the sucrose intake was measured by weighing preweighed bottles containing the sucrose solution, at the end of the test. Subsequently, sucrose consumption was monitored, under similar conditions, at weekly intervals throughout the whole experiment. On the basis of their sucrose intakes in the final baseline test, the animals were divided into two matched groups. One group of animals was subjected to the chronic mild stress procedure for a period of 9 consecutive weeks. Each week of stress regime consisted of: two periods of food or water deprivation, two periods of 45° cage tilt, two periods of intermittent illumination (lights on and off every 2 h), two periods of soiled cage (250 ml water in sawdust bedding), two periods of paired housing, two periods of low-intensity stroboscopic illumination (150 flashes/min), and two periods of no stress. All stressors were 10–14 h of duration, and were applied individually and continuously, day and night. Control animals were housed in separate rooms and had no contact with the stressed animals. They were deprived of food and water for the 14 h preceding each sucrose test, but otherwise food and water were freely available in the home cage.

Drug Administration

The study consisted of two consecutive experiments. In each experiment, both stressed and control animals were further divided into matched subgroups on the basis of sucrose intake scores following 3 weeks of stress. Subsequently, separate groups of control and stressed animals ($n = 8$ rats/group) received once daily intraperitoneal injections of vehicle (1 ml/kg), imipramine (10 mg/kg), and alnespirone (0.5, 1.0, 2.5, and 5.0 mg/kg) in Experiment 1, or vehicle, imipramine (10 mg/kg), and alnespirone (2.5, 5, 10, and 20 mg/kg) in Experiment 2. In both experiments, the drugs were administered at 1000 h, and the weekly sucrose tests were carried out 24 h following the last drug injection. After 5 weeks, all treatments were terminated and one additional sucrose test was carried out following 1 week of withdrawal. Stress was continued throughout the period of treatment and withdrawal.

Drugs

The following agents were used: imipramine (RBI, Natick) and alnespirone (S 20499, Servier). All agents were dissolved in a distilled water that was used for vehicle injections.

Statistics

The results obtained in this study were analyzed by multiple analysis of variance with three between-subjects factors (stress/control, drug treatments, and successive sucrose tests). The Fisher's LSD test was used for post hoc comparisons of means.

RESULTS

In both experiments, CMS caused a gradual decrease in the consumption of 1% sucrose solution. In the final baseline test, all animals drank approximately 15 g of sucrose solution. Following the initial 3 weeks of stress, intakes remained at a similar level in the control animals, but fell to approximately 9 g in the stressed animals, resulting in a highly significant group

effect [Experiment 1: $F(1, 84) = 50.947$, $p < 0.001$; Experiment 2: $F(1, 140) = 217.656$, $p < 0.001$]. In both experiments this difference between control and stressed animals receiving vehicle was maintained for the remainder of the study (see Fig. 1). At the end of the treatment period (week 5) the vehicle-treated stressed animals were slightly smaller than the vehicle-treated controls [mean body weights in Experiment 1: 429 vs. 448 ($p = 0.342$) and in Experiment 2: 408 vs. 452 ($p = 0.048$)].

The effect of imipramine is shown in Fig. 1. In the first experiment, control animals treated with this drug drunk significantly more sucrose solution than those receiving vehicle, $F(1, 84) = 9.189$, $p < 0.01$. However, because imipramine did not significantly increase the intakes over the values measured at week 0 [week effect: $F(5, 42) = 0.686$, NS], this difference appears to be caused by an initial decrease in the consumption of sucrose solution in control animals receiving vehicle rather than by the activity of imipramine itself. Moreover, in the second experiment, imipramine had no significant effect on the consumption of sucrose solution in control animals, $F(1, 84) = 2.649$, NS.

In stressed animals, imipramine gradually increased the sucrose consumption, resulting in a significant treatment effect [Experiment 1: $F(1, 84) = 20.985$, $p < 0.001$; Experiment 2: $F(1, 84) = 10.949$, $p < 0.001$]. The onset of action of imipramine was similar in both experiments; compared to week 0 scores, the effect of this drug in stressed animals reached statistical significance after 4 ($p = 0.05$) and 5 ($p < 0.05$) weeks of treatment. As a result, at the end of the treatment period the amount of sucrose solution drunk by stressed animals receiving imipramine was comparable to that of vehicle-treated controls, and significantly higher than that of vehicle-treated stressed animals (see Fig. 1). These time course and magnitude values for imipramine (as well as other tricyclics) are

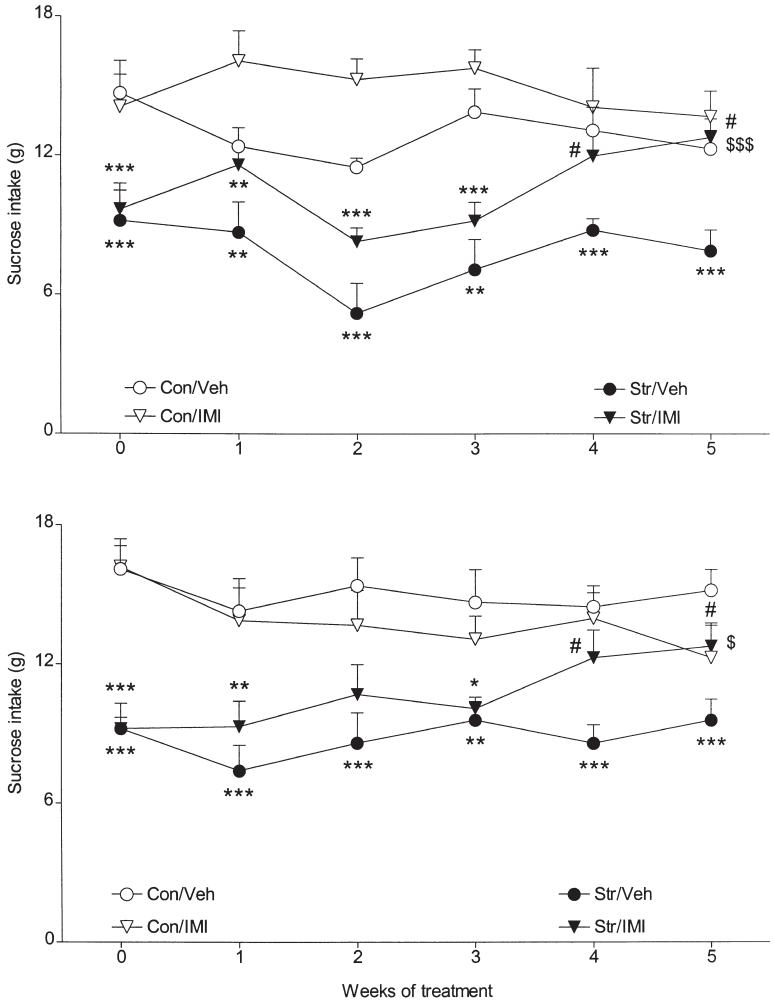

FIG. 1. The effect of chronic treatment with vehicle (Veh, 1 ml/kg/day) and imipramine (IMI, 10 mg/kg/day) on the consumption of 1% sucrose solution in controls (open symbols) and in animals exposed to chronic mild stress (closed symbols). Treatment commenced following 3 weeks of stress. Values are means (±SEM) obtained in Experiment 1 (top panel) and in Experiment 2 (bottom panel). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; relative to vehicle- or drug-treated control groups. $^{\$}p < 0.05$; $^{\$\$\$}p < 0.001$; relative to vehicle-treated stressed animals at week 5. $^{\#}p < 0.05$; relative to drug-treated stressed animals at week 0.

consistent with those obtained in most other studies with the CMS model [see (28,35)].

As shown in Table 1, the imipramine-induced increase of sucrose consumption in stressed animals was maintained at similar level 1 week after withdrawal from the drug (week 5 vs. withdrawal: $p = 0.872$), and the difference between the intakes in stressed animals receiving vehicle and those receiving imipramine remained significant ($p = 0.014$).

The effect of alnespirone in the CMS model is shown in Fig. 2. In both experiments this compound did not significantly affect the consumption of sucrose solution in control animals [Experiment 1: $F(4, 210) = 1.806$, NS; Experiment 2: $F(4, 210) = 0.615$, NS]. However, in stressed animals alnespirone caused a highly significant treatment effects [Experiment 1: $F(4, 210) = 7.102$, $p < 0.001$; Experiment 2: $F(4, 210) = 5.289$, $p < 0.001$].

Analysis of the data obtained in Experiment 1 revealed that, compared to the sucrose intakes in vehicle-treated stressed animals, the lowest dose of alnespirone (0.5 mg/kg/day) was inactive against the CMS-induced deficit in sucrose consumption, $F(1, 84) = 0.006$, NS, and the three higher doses caused a significant and dose-dependent effect [1.0 mg/kg: $F(1, 84) = 6.012$, $p < 0.05$; 2.5 mg/kg: $F(1, 84) = 7.113$, $p < 0.01$; 5 mg/kg: $F(1, 84) = 15.886$, $p < 0.001$]. However, when compared to the sucrose intakes at week 0, only the highest dose of alnespirone (5 mg/kg/day) significantly increased the consumption of sucrose solution at the end of the 5 weeks of treatment period ($p = 0.013$). The lack of significant effects in animals receiving alnespirone at the doses of 1.0 and 2.5 mg/kg/day could result from relatively high SEM values; approximately two animals in each group did not respond to the treatment. Nevertheless, after 5 weeks of treatment with these doses of alnespirone, the stressed animals drank significantly more sucrose solution than the stressed animals receiving vehicle (see Fig. 2, top panel).

Out of four doses of alnespirone tested in Experiment 2, only the lowest one (2.5 mg/kg/day) was able to fully reverse the CMS-induced deficit in sucrose consumption. In stressed animals treated with this dose the sucrose intake was significantly increased from initial scores (week 0) after 4 ($p < 0.05$) and 5 ($p < 0.01$) weeks, and at the end of treatment period the intakes did not significantly differ from those of vehicle-treated controls, and were significantly higher than those of vehicle-treated stressed animals (see Fig. 2, bottom panel). The stressed animals receiving 5 mg/kg/day of alnespirone also improved their sucrose consumption, $F(1, 84) = 5.309$, $p < 0.05$, but the post hoc comparison of means revealed that this effect did not reach statistical significance at any time point. Nevertheless, consistently with the results of the Experiment 1, after 5 weeks of treatment with 5 mg/kg/day of alnespirone, the stressed animals drank significantly more sucrose solution than the stressed animals receiving vehicle (see Fig. 2, bottom panel). Finally, the two highest doses of alnespirone (10 and 20 mg/kg/day) were ineffective against the CMS-induced deficit in sucrose intakes, $F(2, 126) = 0.308$, NS.

As was observed in imipramine-treated rats, the effect of alnespirone was maintained at similar level 1 week after withdrawal from the treatment in the stressed groups, and the sucrose consumption was not significantly altered in all alnespirone-treated control animals [Experiment 1: $F(3, 56) = 0.622$, NS; Experiment 2: $F(3, 56) = 0.018$, NS; Table 1].

In control animals only imipramine in the second experiment caused significant weight loss, $F(1, 14) = 7.474$, $p < 0.05$. In stressed animals the body weights were significantly decreased by imipramine in Experiment 1, $F(1, 14) = 6.572$, $p < 0.05$, but not in Experiment 2, $F(1, 14) = 0.529$, NS, and alnespirone had no significant effect on the body weights in both experiments [Experiment 1: $F(4, 35) = 0.375$, NS; Experiment 2: $F(4, 35) = 0.380$, NS.

TABLE 1

SUCROSE CONSUMPTION IN STRESSED ANIMALS FOLLOWING 5 WEEKS OF TREATMENT AND ONE WEEK OF WITHDRAWAL

Treatment	Week 5	Withdrawal
Vehicle	8.6 ± 0.9	7.8 ± 1.3
Imipramine	12.8 ± 1.0	13.1 ± 1.6
Alnespirone		
0.5 mg/kg	8.5 ± 0.8	11.1 ± 1.0
1.0 mg/kg	10.9 ± 1.1	11.4 ± 1.6
2.5 mg/kg	11.3 ± 1.4	11.5 ± 1.6
5.0 mg/kg	13.1 ± 1.0	12.0 ± 0.7
Alnespirone		
2.5 mg/kg	13.4 ± 1.2	12.1 ± 1.3
5.0 mg/kg	12.0 ± 1.5	11.4 ± 1.5
10 mg/kg	9.9 ± 1.4	9.3 ± 1.5
20 mg/kg	10.0 ± 0.9	8.2 ± 1.1

Values are means ± SEM. No significant differences were found between week 5 and withdrawal values in any treatment groups. See text for details.

DISCUSSION

The present study demonstrates that chronic administration of alnespirone, the selective and potent agonist of the 5-HT_{1A} receptor agonist, can reverse the reduction in the consumption of a 1% sucrose solution produced by CMS. This finding is consistent with the effects of alnespirone in other animal procedures predictive of antidepressant action, such as learned-helplessness, unavoidable light-stimulus, and olfactory bulbectomy tests (17,20,21).

The action of active doses of alnespirone in the CMS model appears to be similar to that of imipramine. Thus, the increases in sucrose intakes were observed in animals subjected to the CMS procedure, and the behavior of nonstressed control groups was not affected by any doses of alnespirone tested in this study. This is a characteristic feature of the action of all antidepressant drugs tested in the CMS model, and corresponds to the failure of these drugs to elevate mood in nondepressed human subjects (29). The lack of effect of alnespirone on the consumption of sucrose solution in nonstressed control animals is consistent with other preclinical and clinical toxicological studies demonstrating that this compound is devoid of any effect on water consumption. Also the time course of action of alnespirone in the CMS model and the magnitude of this effect were comparable to those of imipramine; 4–5 weeks of treatment with the doses of 2.5 and 5 mg/kg/day were required to produce the first significant increase in sucrose intakes, and at the end of the treatment period a full recovery from the CMS-induced behavioral deficit was observed in both imipramine- and alnespirone-treated stressed animals. All these findings confirm previous reports showing that alnespirone causes antidepressant-like activity that is comparable to that of imipramine in studies where this drug was used as the reference treatment, i.e., learned-helplessness and unavoidable light-stimulus tests (17,21). Moreover, as found in the present study, the effect of alnespirone

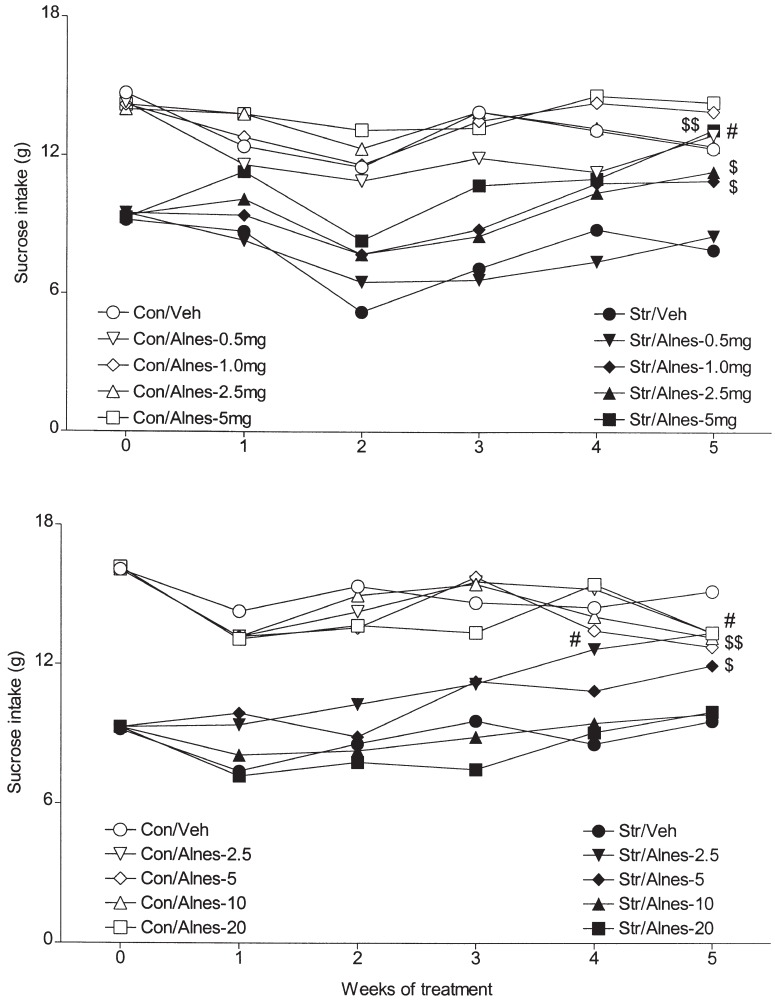

FIG. 2. The effect of chronic treatment with vehicle (Veh, 1 ml/kg/day) and alnespirone (Alnes, 0.5–20 mg/kg/day) on the consumption of 1% sucrose solution, in controls (open symbols) and in animals exposed to chronic mild stress (closed symbols). Treatment commenced following 3 weeks of stress. Values are mean (SEM has been omitted for clarity) obtained in Experiment 1 (top panel) and in Experiment 2 (bottom panel). See text for further statistical details. $^{\$}p < 0.05$, $^{\$\$}p < 0.01$; relative to vehicle-treated stressed animals at week 5. $\#p < 0.05$; relative to drug-treated stressed animals at week 0.

and imipramine in rats subjected to the CMS procedure was maintained for at least 1 week after cessation of the treatment, and no signs of withdrawal were observed in both stressed and control animals. The lack of a withdrawal syndrome following discontinuation of chronic alnespirone administration has been also found in other studies with this compound (13).

The effect of alnespirone in the CMS model has an inverted U-shaped dose–response pattern; the significant effects on the stress-induced deficit in the consumption of sucrose solution were observed in animals receiving this compound at the midrange doses (1–5 mg/kg/day), and the lower or higher doses were inactive. A similar U-shaped dose–response relationship was also reported in the learned-helplessness and unavoidable light-stimulus tests (17,21), and is consistent with the in vivo characterization of alnespirone as a full presynaptic but only partial postsynaptic 5-HT_{1A} receptor agonist (see the introduction). The inefficacy of lower doses (i.e. <1 mg/kg/day) of alnespirone in the CMS model of depression is not surprising, because at this dose the compound shows potent anxiolytic activity (1,6,12,14,30), which does not seem to be responsible for the reduction of the stress-induced deficits in sucrose consumption (23). However, the reason for loss of efficacy of higher doses (i.e., >10 mg/kg/day) found both in the CMS model and in learned-helplessness and unavoidable light-stimulus tests, is not clear, and requires further studies.

It has recently been suggested that the CMS-induced deficit in sucrose consumption and its restoration by chronic drug treatments may be secondary to changes in animals body weights (19). However, as evaluated and discussed fully elsewhere (34,35), in this study the decrease of sucrose intake also cannot be attributed to decrements in body weights, which in

stressed animals receiving vehicle were absent in Experiment 1 and relatively small in Experiment 2. Similar dissociation between sucrose intakes and body weights was observed in drug-treated animals; imipramine normalized the CMS-induced decrease in sucrose intakes in both experiments but caused significant loss of weight only in Experiment 1, and alnespirone had no significant effect on body weights in either experiments.

The mechanism of reversal of CMS-induced anhedonia has been suggested to involve an increase in dopamine function because agonists of D_2/D_3 receptors are active in the CMS model (24,26), and these receptors are involved in both the CMS-induced deficit in sucrose consumption and in the therapeutic activity of imipramine (10,22,23,27,32). Our results with alnespirone indicate that other neurochemical mechanisms may also be involved because it has been shown in electrophysiological and neurochemical studies that alnespirone does not affect dopaminergic systems at doses that induce anxiolytic or antidepressant effects in rats; only at higher doses (34 mg/kg IP) an increase in dopamine turnover can be observed, but this effect appears to result from the 5-HT_{1A} receptor stimulation rather than from direct action of alnespirone on dopaminergic receptors (9). Buspirone, which is pharmacologically similar to alnespirone, also shows activity in this model (31). Another 5-HT_{1A} receptor ligand, S 15535, which acts as an agonist of presynaptic and an antagonist of postsynaptic 5-HT_{1A} receptors, failed to reverse the CMS-induced anhedonia (data not shown). Because in vivo alnespirone behaves as a full presynaptic and partial postsynaptic 5-HT_{1A} agonist, this result would suggest that the postsynaptic—rather than presynaptic—receptors are involved in the antidepressant action of drugs in the CMS model of depression. This implication is consistent with the role attributed to the postsynaptic receptors in the antidepressant effects of 5-HT_{1A} agonists (18). In this context it should, however, be noted that in previous studies with the CMS model the full presynaptic and postsynaptic 5-HT_{1A} agonist, 8-OH-DPAT, was inactive against the stress-induced anhedonia (31). We cannot offer any reasonable explanation for these apparent discrepancy, but similar differences between behavioral effects of alnespirone and 8-OH-DPAT were also found in other studies. For example, alnespirone, unlike 8-OH-DPAT, is unable to produce the "5-HT_{1A} syndrome" and other behaviors that are known to result from the stimulation of postsynaptic 5-HT_{1A} receptors (33). Also, in recent binding studies it was found that, although the regional distribution of specific binding sites of alnespirone match those of 8-OH-DPAT, both compounds apparently do not interact in the same way with 5-HT_{1A} postsynaptic receptors (11). The exact nature and mechanisms of these differences are not clear, but it is suggested that it may reflect a variability in efficacy from one cell type to and another, which in consequence, can result in differences in behavioral effects of the two 5-HT-$_{1A}$ agonists.

In conclusion, the finding that the potent and selective agonist of 5-HT_{1A} receptors can normalize the behavioral deficit in the animal model of depression with a high degree of face, construct, and predictive validity [see (35)] provides further support for the hypothesis that these receptors may be involved in the mechanisms of depression, and that their activation may indeed result in antidepressant activity. The fact that other agonists at both pre- and postsynaptic 5-HT_{1A} receptors such as 8-OH-DPAT and ipsapirone, do not show any activity in the CMS paradigm (31) implies that further investigations are needed to clarify the role of pre- and postsynaptic 5-HT_{1A} receptors and their respective ligands in this model of depression.

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