Selective Antiaggressive Effects of Alnespirone in Resident-Intruder Test Are Mediated via 5-Hydroxytryptamine$_{1A}$ Receptors: A Comparative Pharmacological Study with 8-Hydroxy-2-Dipropylaminotetralin, Ipsapirone, Buspirone, Eltoprazine, and WAY-100635

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Accepted for publication October 6, 1998 This paper is available online at http://www.jpet.org

ABSTRACT

The present study characterized the effects of the novel, selective, and potent 5-hydroxytryptamine$_{1A}$ (serotonin) (5-HT$_{1A}$) receptor agonist, alnespirone [S-20499, (S)-N-4-[5-methoxychroman-3-yl]propylamino)butyl-8-azaspiro-(4,5)-diacetamide, hydrochloride] on offensive and defensive resident-intruder aggression in wild-type rats and compared its actions with those of the prototypical full 5-HT$_{1A}$ agonist 8-hydroxy-2-dipropylaminotetralin (8-OH-DPAT), the partial 5-HT$_{1A}$ agonists ipsapirone and buspirone, and the mixed 5-HT$_{1A}$/1B agonist eltoprazine. All five agonists exerted effective dose-dependent decreases of offensive aggressive behavior in resident rats; 8-OH-DPAT was the most potent (ID$_{50}$ 0.074 mg/kg), followed by eltoprazine (0.24), buspirone (0.72), ipsapirone (1.08), and alnespirone (1.24). However, in terms of selectivity of the antiaggressive effects as determined by the absence of decrements in social interest and general motor activity, alnespirone appeared to be superior. In the defensive aggression test, neither alnespirone nor any of the other four agonists changed defensive behaviors in the intruder rats. The involvement of 5-HT$_{1A}$ receptors in the antiaggressive actions of these drugs was confirmed by showing that the selective 5-HT$_{1A}$ receptor antagonist WAY-100635 [N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride], which was inactive alone, fully prevented the antiaggressive effects of alnespirone, 8-OH-DPAT, and buspirone and partly reversed those of ipsapirone and eltoprazine. The data clearly indicate that alnespirone effectively suppresses offensive aggression with an advantageous profile of action compared with other full or partial 5-HT$_{1A}$ agonists. These selective antiaggressive actions of alnespirone are mediated by stimulating 5-HT$_{1A}$ receptors, presumably the somatodendritic autoreceptors at the raphe nuclei. Furthermore, the data provide evidence for a major involvement of these 5-HT$_{1A}$ receptors in the modulation of aggressive behavior by 8-OH-DPAT, ipsapirone, buspirone, and eltoprazine.

Alnespirone [S-20499, (S)-N-4-[5-methoxychroman-3-yl]propylamino)butyl-8-azaspiro-(4,5)-diacetamide, hydrochloride] is a novel amino chroman derivative with potent and selective agonist properties at central 5-hydroxytryptamine$_{1A}$ (serotonin) (5-HT$_{1A}$) receptors (Kidl et al., 1993; Scott et al., 1994; Casanovas et al., 1997; Fabre et al., 1997). Accordinly, because brain 5-HT$_{1A}$ receptors are implicated in the pathophysiology of anxiety and depression (Crapol et al., 1995), alnespirone has reliably been reported to display anxiolytic and antidepressant properties in a variety of animal behavioral models (Griebel et al., 1992; Porsolt et al., 1992; Barrett et al., 1994; Curle et al., 1994; File and Andrews, 1994; MacSweeney et al., 1998). Besides the involvement in anxiety and depression, serotonergic receptors of the 5-HT$_{1A}$ type are particularly implicated in aggressive behavior. Drugs that selectively activate this receptor site are among the most promising novel therapeutic agents for altering excessive violent and aggressive behavior (Ratey et al., 1991; Miczek et al., 1995; Olivier et al., 1995). Stimulation of the 5-HT$_{1A}$ receptor by more or less selective partial and full agonists, such as buspirone, gepirone, ipsapirone, 8-hydroxy-2-dipropylaminotetralin (8-OH-DPAT), fluprazine, eltoprazine, and flesinoxan results in potent antiaggressive effects across a range of animal species and test situations (Tompkins et al., 1980; Flannely et al., 1985; Lindgren and Kantak, 1987; Blanchard et al., 1988; McMillen et al., 1988; Nikulina, 1991; Bell and Hobson, 1994; Bonson et al., 1994;}

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine (serotonin); 8-OH-DPAT, 8-hydroxy-2-dipropylaminotetralin; ALT, attack latency time; ID, inhibitory dose; WAY-100635, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride.

Received for publication May 28, 1998.
Olivier et al., 1994; Sanchez et al., 1996; Sanchez and Hyttel, 1994; Miczek et al., 1995; Muehlenkamp et al., 1995, 1996). However, with the noticeable exception of eltoprazine and fluprazine, the profound antiaggressive effects of these drugs coincide with and probably are secondary to the behaviorally nonspecific incapacitating effects these drugs exert, such as sedation, motor debilitation, and inhibition of the entire social behavioral repertoire (i.e., the “5-HT syndrome”): Tricklebank, 1985). After a comparison of a variety of 5-HT receptor agonists in a number of aggression studies (Mos et al., 1992, 1993; Sijbesma et al., 1991), Olivier et al. (1995) concluded that the specific reduction of offensive aggression (or “serenic activity”) by eltoprazine and fluprazine is mediated via postsynaptically located 5-HT1A binding sites. However, in all these studies, a contribution of the 5-HT1A receptor could not be ruled out because of the lack of good selective 5-HT1A receptor antagonists at the time.

A first experiment by File and Andrews (1994) using the social interaction test in male Lister rats provided some indications that alnespirone, in addition to increasing social investigation (indicating anxiolytic-like actions), had the effect of reducing aggressive behaviors. Because the social interaction test is not specifically aimed at demonstrating antiaggressive effects of drugs, a more extensive ethological analysis in an animal model for agonistic behavior is required to determine how selectively alnespirone affects aggressive behavior in the rat (see, for example, Miczek et al., 1995). Therefore, in this study, the antiaggressive effects of alnespirone were studied in a resident-intruder paradigm, which has the possibility to test the effects of the drug on offensive aggression (in the resident initiated by the intrusion of an unfamiliar male into its home territory) as well as on defensive aggression (in the intruder). Moreover, by making an extensive recording of the behavior in this test, it was possible to determine the selectivity of the drug effects for aggression or nonspecific (i.e., sedative) effects (see Olivier et al., 1995; Miczek et al., 1989, for reviews of the various kinds of aggressive behavior and use of different animal models). The effects of alnespirone in this paradigm were compared with those of the prototypical full 5-HT1A agonist 8-OH-DPAT, the partial 5-HT1A agonists ipsapirone and buspirone, and the mixed 5-HT1A/1B agonist eltoprazine. Furthermore, the involvement of 5-HT1A receptors in the putative antiaggressive actions of these drugs was assessed by using the selective and silent 5-HT1A antagonist WAY-106635 (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclohexane-carboxamide trihydrochloride) (Forster et al., 1995; Fletcher et al., 1996).

**Materials and Methods**

**Subjects and Housing.** A total of 527 male Wild Type Groningen (WTG) rats (Rattus norvegicus; originally wild-trapped animals and bred for approximately 17 generations in our own laboratory under specific-pathogen-free conditions) that were 3.5 months old were used as experimental subjects. This strain is preferred for agonistic studies because they exhibit an easy to evoke and rich natural repertoire of intraspecific aggressive and social behaviors. They were housed in clear Plexiglas cages (60 × 60 × 20 cm) in groups of five or six from weaning (23 days after birth) until the start (at age 140 days) of the experiments. The cages were placed in a temperature-controlled room (22 ± 2°C) with a fixed 12-h light/dark photoperiod (lights off at 1:00 pm). The animals were allowed free access to water and food (Hope Farms Lab Chow).

**Experimental Procedures.** A resident-intruder agonistic paradigm was used to monitor either offensive behavior (experimental resident) or defensive behavior (experimental intruder) that strongly resembles the natural patterns of wild rats to establish and defend their territory (Koolhaas et al., 1980). In the resident-intruder offensive model, the animals were housed individually in observation cages (80 × 55 × 50 cm), each with a sterilized female to avoid social isolation and to facilitate territorial behavior. After 1 week, the baseline level of offensive behavior was tested on 3 consecutive days during a 10-min confrontation with an unfamiliar male conspecific in the home territory of the experimental (resident) rat. These naive intruder-rats were socially housed in groups of five or six animals in clear Plexiglas cages (60 × 60 × 20 cm). Approximately 1 h before the start of the confrontation, the female of the experimental rat was removed from the observation cage. Experimental groups were balanced on the basis of offensive behavior performed during the third baseline test, during which the full range of behavioral elements was recorded (see below). Animals that showed less than 10% offensive behavior (ALT greater than 500 s) were not included in the drug treatment tests (approximately 15% of the animals).

On the next day, 30 min before the 10-min confrontation with an intruder, the experimental resident rats received one dose of one of the following s.c. injections (i.e., animals were tested only once): vehicle (1 ml/kg distilled water) alnespirone (0.5, 1, 5, and 10 mg/kg), 8-OH-DPAT (0.05, 0.1, 0.25, 0.5, and 1.0 mg/kg), buspirone (0.25, 0.5, 1.0, and 5.0 mg/kg), ipsapirone (0.5, 1.0, and 5.0 mg/kg), or eltoprazine (0.1, 0.25, 0.5, 1.0, and 2.5 mg/kg). During the 10-min confrontation with an unfamiliar and undrugged conspecific intruder, the full range of behaviors was recorded again.

In case of the antagonism studies, vehicle (distilled water) or WAY-106635 (0.01, 0.1, or 1.0 mg/kg) was administered 15 min before single challenge doses of either vehicle, alnespirone (5.0 mg/kg), 8-OH-DPAT (0.1 mg/kg), buspirone (2.5 mg/kg), ipsapirone (5 mg/kg), and eltoprazine (1 mg/kg), and 30 min later, the agonistic behavior of the drugged resident rats was examined by ethological procedures during a 10-min social encounter with an undrugged intruder. The selected doses of the 5-HT1A agonists were based on submaximal effective dosages to inhibit aggressive behavior found previously in the dose-response study.

In the resident-intruder defensive model, another group of experimental animals served as naive intruders into the home territory of a well trained (5–10 consecutive successful winning experiences) aggressive resident counterpart. The ensuing agonistic interaction was accompanied by a variety of defensive body postures and escape behaviors of the experimental intruder rat that can be recorded. Therefore, this model gives the opportunity to assess the effects of the 5-HT1A agonists on the complete natural defensive behavioral repertoire. Alnespirone (1, 5, and 10 mg/kg), 8-OH-DPAT (0.1, 0.25, and 0.5 mg/kg), buspirone (0.5 and 5 mg/kg), ipsapirone (0.5 and 5 mg/kg), or eltoprazine (0.5 and 1 mg/kg), or vehicle (distilled water) was administered s.c. 30 min before placement of the experimental animal into the home territory of an aggressive male resident for 10 min. The selected doses of the 5-HT1A agonists were based on effective dosages to inhibit aggressive behavior found previously in the dose-response study.

During the agonistic confrontations, the full range of behaviors of either the experimental resident rat (offensive aggression test) or the experimental intruder rat (defensive aggression test) was recorded on videotape and manually scored on a keyboard processor. An extensive description of the different behavioral elements displayed during agonistic interactions has been reported previously (Koolhaas et al., 1980). Briefly, the following behavioral elements were distinguished: 1) lateral threat; 2) keep down; 3) clinch; 4) chase/flight; 5) offensive/defensive upright; 6) investigating opponent/moving toward; 7) ambulation, scanning, digging; 8) rearing; 9) grooming; 10) social grooming/crawl over; 11) ano-genital sniffing; 12) inactivity;
13) freeze/crouch; 14) submissive posture; 15) (attempt to) mounting; and 16) keep off. The duration of the different behavioral elements was computed and expressed as a percentage of the total duration (10 min) of the confrontation. To promote a clear representation of the data, the elements lateral threat, offensive upright, keep down, clinch, and chase were taken as one behavioral category (i.e., “offensive behavior”), whereas the term “defensive behavior” is used for submissive posture, defensive upright, flight, freeze, and keep off. Also, the latency time to the first attack (“attack latency time”) by the resident was taken as a measure of aggressiveness. The term “social explorative behavior” is used for the elements investigating opponent and anogenital sniffing, whereas “social contact behavior” encompasses the elements social grooming, crawl over, and mounting. “Nonsocial explorative behavior” is used for the elements ambulation and rearing.

**Drugs.** Alnespirone [(+)-8-20499-2 hydrochloride; lot no. 45109; molecular weight 479] was provided by Institut de Recherches Internationales (Servier, France). WAY-100635 [N-[2-[4-(2-methoxy-phenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride; lot no. A-05; molecular weight 513] was a gift from Wyeth Research (UK) Ltd. (±)-8-OH-DPAT hydrobromide (molecular weight 328) and buspirone hydrochloride (molecular weight 422) were obtained from Research Biochemicals International (Natick, MA). Ipsapirone hydrochloride (molecular weight 438) and etizolam dihydrochloride (molecular weight 258) were kindly provided by Solvay Duphar Pharmaceuticals (Weep, The Netherlands). All drugs were freshly dissolved in sterile distilled water (vehicle solution) and pH adjusted to as close to neutrality as possible. Solutions were prepared approximately 1 h before the start of the experiments. The injections were given s.c. in the flank region in a volume of 1 ml/kg b.wt.

**Data Analysis.** Data are expressed as mean ± S.E.M. The dose-effect curves for each behavioral catagory and attack latency time were analyzed by a one-way ANOVA, with drug dose as between-subject factor. In the dose-response studies, the drug effects on each behavioral catagory were also computed as a percentage of the respective vehicle control values to enable a comparison between the various drugs. Least-squares linear regression analysis was used to estimate the dose (mg/kg) that would elicit 50% aggression reduction (ED50) and the corresponding 95% confidence limits. ED50 values with 95% confidence limits that did not overlap were considered to be statistically different. In the antagonist study, the drug-effect histograms for each behavioral category and attack latency time were analyzed by a two-way ANOVA, with pretreatment as between-subject factor 1 (two levels: vehicle and WAY 0.1) and drug as between-subject factor 2 (six levels). Further analyses were made by Duncan’s new multiple-range test (between-subject effects) to determine the source of detected significance in the ANOVAs. The criterion of significance was set at $P < .05$.

**Results**

**Offensive Aggression Test: General Aspects and Dose-Response Effects.** Social confrontation initiated by the intrusion of an unfamiliar male rat into the home cage of the territorial experimental male counterpart resulted in a typical offensive aggressive behavioral pattern of the resident, consisting of an approach to and pursuing of the intruder, followed by anogenital sniffing (sometimes followed by mounting attempts), and a threaten/attack sequence resulting in clinching, biting, chasing, and forcing the intruder into submission. The latency time to the first attack (clinch) in undrugged residents ($n = 223$) ranged from 6 to 461 s with a mean of 125 ± 8.2 s. These fights always resulted in defeat of the intruder rat, which exhibited a variety of defensive/submissive body postures and escape responses. Characteristically, several bouts of fighting alternate with periods of no agonistic interactions during the observation trial. During the 10-min agonistic encounters on day 1, undrugged resident rats spent $43.1 ± 1.7\%$ of the time on offensive aggressive behavior and $12.9 ± 0.7\%$ on total social explorative behavior, thus spending $56.1 ± 1.5\%$ on total social interaction. In the remainder of the 10-min observation period, animals spent $33.9 ± 1.3\%$ of the time on nonsocial exploration, $2.5 ± 0.3\%$ on grooming, and $7.0 ± 0.7\%$ on inactivity. A roughly similar behavioral pattern was observed the next day in the group of rats injected with vehicle (Fig. 1), but compared with the undrugged condition, the vehicle-injected animals spent significantly less time on offensive and social explorative behavior and more time on inactive and grooming behaviors.

Compared with vehicle treatment, alnespirone-treated rats showed a significant, dose-dependent delay in the latency time to attack (Fig. 2, inset) and reduction in the amount of offensive behavior (Fig. 2) toward the intruder rat. This reduction in offensive behavior was accompanied by a significant increase in social explorative behavior, thereby leaving total social interaction time in the alnespirone-drugged rats as the same. In addition, the drug did not significantly modify the nonsocial activities of exploration, grooming, or inactivity (Fig. 2).

8-OH-DPAT-treated rats showed a pronounced, dose-dependent reduction in offensive aggressive behavior (Fig. 3) as well. However, this antiaggressive effect was accompanied by a pronounced increase in social explorative behavior, thereby leaving total social interaction time in the alnespirone-drugged rats as the same. In addition, the drug did not significantly modify the nonsocial activities of exploration, grooming, or inactivity (Fig. 3).

8-OH-DPAT-treated rats showed a pronounced, dose-dependent reduction in offensive aggressive behavior (Fig. 3) as well. However, this antiaggressive effect was accompanied by a pronounced increase in social explorative behavior. Starting from the 0.1 mg/kg dose, 8-OH-DPAT induced clear signs of the full serotonergic syndrome, especially characterized by flat body-posture, head-waving, forepaw treading, and hindlimb abduction, leading to increased behavioral inactivity scores. Because social exploration was not affected by 8-OH-DPAT, total social interaction decreased significantly after 8-OH-DPAT treatment.

Similar to the behavioral profile induced by 8-OH-DPAT, buspirone- (Fig. 4) and ipsapirone- (Fig. 5) treated animals showed a potent, dose-dependent reduction in aggression that was accompanied by significant increases in behavioral inactivity. The highest dose of each drug (5.0 mg/kg) pro-

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**Fig. 1.** Mean ± S.D. percentages of time spent on six distinct behavioral catagories and mean attack latency scores (inset) during offensive aggression test on day 1 (undrugged condition; gray bars) and day 2 (vehicle-treated; black bars). *Values are significantly (at least $p < .05$, Student’s t test) different from undrugged condition value ($n = 58$).
duced some of the signs of the serotonergic syndrome (flat body posture).

Eltoprazine treatment (Fig. 6) also produced a potent dose-dependent inhibition of aggressive behavior, which was accompanied by an increase in both social exploration and inactivity. Although there was a significant enhancement of social exploration, this did not fully compensate for the decrease in aggression, and thus total social interaction decreased significantly with the two highest doses of eltoprazine. It was noted that the two lowest doses (0.25 and 0.5 mg/kg) of eltoprazine that significantly decreased aggression, and thus total social interaction decreased significantly with the two highest doses of eltoprazine, but no increase after any dose of alnespirone.

The introduction of a vehicle-treated experimental rat into the home territory of an experimental intruder. The socially defeated intruder exhibited immediately a variety of defensive body postures and escape behaviors, including flight, freezing, defensive upright, keep off, and submission. Vehicle-treated rats spent 64.2 ± 4.3% of the observation time on active avoidance, whereas the rats treated with the 8-OH-DPAT (ID$_{50}$ = 0.24 mg/kg) were significantly different from each other as well as from buspirone (ID$_{50}$ = 0.72 mg/kg), ipsapirone (ID$_{50}$ = 1.08 mg/kg), and alnespirone (ID$_{50}$ = 1.24 mg/kg), whereas the differences between alnespirone, ipsapirone, and buspirone were not significantly different. As noted before, Fig. 7 also more clearly shows the different qualitative and quantitative abilities of the five agonists to increase behavioral inactivity: a potent and pronounced dose-dependent increase after 8-OH-DPAT, followed by buspirone, ipsapirone, and eltoprazine, but no increase after any dose of alnespirone.

**Defensive Aggression Test.** The introduction of a vehicle-treated experimental rat into the home territory of an undrugged, trained aggressive resident counterpart resulted in an agonistic interaction (mean attack latency time of the resident = 88.8 s; see Table 1), leading to a rapid defeat of the experimental intruder. The socially defeated intruder exhibited immediately a variety of defensive body postures and escape behaviors, including flight, freezing, defensive upright, keep off, and submission. Vehicle-treated rats spent 64.2 ± 4.3% of the observation time on defensive behavior (submissive posture, defensive upright, flight, freeze, and...
Within the dose-ranges tested, alnespirone-, 8-OH-DPAT-, buspirone-, ipsapirone-, and eltoprazine-treated rats showed a similar defensive behavioral pattern as vehicle-treated rats when exposed to an aggressive resident (Table 1). In addition, the drug-treated intruders were not attacked more often or vigorously than the vehicle-treated counterparts, nor was their other behavioral repertoire affected by the drugs. The observed unchanged aggressive behavior of the resident attacker toward drug-treated intruders also indicates that the behavior of the latter is not dramatically changed. With the highest dose of 8-OH-DPAT, buspirone, and ipsapirone, however, clear signs of flat body posture appeared, but once defeated by the resident rat, this behavioral response was indistinguishable from the exhibited submissive and freezing behavior.

WAY-100635 Pretreatment. Pretreatment with the selective 5-HT_{1A} antagonist WAY-100635 (0.01, 0.1, and 1.0 mg/kg) did not result in overall significant behavioral changes of the vehicle-treated group of rats (Fig. 8). One-way ANOVA with drug dose as between-subject factor did not reveal significant effects for any of the behavioral parameters. Nevertheless, at the individual level, the drug seems to decrease offensive behavior in animals with very high (greater than 50%) levels of aggressiveness and to increase it in animals with low (less than 15%) levels of aggressiveness. WAY-100635 (0.1 mg/kg) almost completely blocked the antiaggressive effects (enhanced ALT, decreased offensive behavior) induced by submaximal doses of alnespirone, 8-OH-DPAT, and buspirone and partly antagonized the antiaggressive effects of ipsapirone and eltoprazine (Fig. 8). A higher dose of WAY-100635 (1 mg/kg) also was not able to fully block the antiaggressive effects of ipsapirone and eltoprazine. Interestingly, however, the significantly enhanced behavioral inactivity observed after treatment with 8-OH-DPAT, buspirone, ipsapirone, and eltoprazine was reversed with the WAY-100635 pretreatment.

**Discussion**

The present findings clearly demonstrate that 1) the selective 5-HT_{1A} receptor agonist alnespirone dose-dependently suppresses offensive aggressive behavior without the disruptive effects on (social) exploration and motor activity typically occurring after 8-OH-DPAT, buspirone, and ipsapirone and 2) the antiaggressive effects are prevented by the selective 5-HT_{1A} antagonist WAY-100635, providing evidence that 5-HT_{1A} receptors are mediating this effect of alnespirone, 8-OH-DPAT, buspirone, ipsapirone, and eltoprazine. The antiaggressive profile of alnespirone was characterized by a strong reduction (both in frequency and duration) in resident rat attacks and pursuits toward an intruder and accompanied by a compensatory increase in normal social explorative and contact behavior (mainly mounting attempts), thereby not affecting total social interaction scores. This observation is in agreement with the previously reported lack of effect of alnespirone on social anxiety in the social interaction test using Lister rats (File and Andrews, 1994), where alnespirone increased social investigation but at the same time decreased aggression. These dose-dependent changes in the final consummatory parts of the offensive behavioral repertoire are not secondary to any general depressant or motor-incapacitating effects of alnespirone because neither nonsocial exploration and behavioral inactivity (sitting, lying, immobility) nor the defensive behavioral repertoire was modified by this drug. Thus, the antiaggress-
sive effect of alnespirone seems to be very specific in that it is dose dependent, does not impair normal social interactions and defense/flight abilities, and is without any unwanted motor effects like general sedation and muscle relaxation.

This specific antiaggressive profile of alnespirone is profoundly different from that of all the other tested full or partial 5-HT1A agonists such as 8-OH-DPAT, buspirone, and ipsapirone, which potently decrease offensive aggression at doses while strongly reducing social exploration and motor activity. These observations of rather unselective antiaggressive effects of 8-OH-DPAT, buspirone, and ipsapirone are in line with what has been reported before by several authors using various aggression paradigms (Tompkins et al., 1980; Flannely et al., 1985; Lindgren and Kantak, 1987; Blanchard et al., 1988; McMillen et al., 1988; Nikulina, 1991; Bell and Hobson, 1994; Olivier et al., 1994, 1995; Sanchez and Hyttel, 1994; Miczek et al., 1995; Muehlenkamp et al., 1995). The reduction in offensive aggression by the full 5-HT1A agonist 8-OH-DPAT and partial agonists buspirone and ipsapirone are generally explained by the primary sedating/motor-inactivating effects these compounds induce as part of the well described 5-HT behavioral syndrome (Tricklebank, 1985; Scott et al., 1994; Millan et al., 1994).

The behavioral profile of alnespirone closely resembles that observed in a similar test situation by Olivier et al. (1989) for the “serenics” eltoprazine (mixed 5-HT1A/B agonist), fluprazine, and TFMPP (weak 5-HT1A/B/C and 5-HT2c agonists). In an extensive and well conducted series of experiments (Tompkins et al., 1980; Flannely et al., 1985; Lindgren and Kantak, 1987; Blanchard et al., 1988; McMillen et al., 1988; Nikulina, 1991; Bell and Hobson, 1994; Olivier et al., 1994, 1995; Sanchez and Hyttel, 1994; Miczek et al., 1995; Muehlenkamp et al., 1995). The reduction in offensive aggression by the full 5-HT1A agonist 8-OH-DPAT and partial agonists buspirone and ipsapirone are generally explained by the primary sedating/motor-inactivating effects these compounds induce as part of the well described 5-HT behavioral syndrome (Tricklebank, 1985; Scott et al., 1994; Millan et al., 1994).

The behavioral profile of alnespirone closely resembles that observed in a similar test situation by Olivier et al. (1989) for the “serenics” eltoprazine (mixed 5-HT1A/B agonist), fluprazine, and TFMPP (weak 5-HT1A/B/C and 5-HT2c agonists). In an extensive and well conducted series of experiments, it was reported that these drugs specifically and effectively reduce offensive aggression, leaving defense and other activities intact. In this study, eltoprazine in the lower dose-range also exerted very potent antiaggressive effects without clear signs of sedation/motor dysfunctions, but with higher dosages, significant increases of behavioral inactivity did occur. The fact that the antiaggressive effect of eltoprazine at higher doses was also associated with a significant increase of behavioral inactivity seems, at first sight, to contrast with data from Olivier and associates. However, with scrutiny of the results of a number of their studies, they clearly show that the reduction in aggressive behavior by eltoprazine is accompanied by enhanced inactivity scores, which after the highest doses of eltoprazine even reach statistical significance (Mos et al., 1993; Olivier et al., 1994).

Furthermore, after an extensive series of experiments aimed at determining the 5-HT site and mechanism of action, it was concluded by Olivier et al. (1995) that the specific antiaggressive properties of eltoprazine are most likely mediated via activation of postsynaptic 5-HT1B binding sites (Sjibesma et al., 1991; Mos et al., 1992, 1993), although a contribution from the 5-HT1A site could not be ruled out. The view of a predominant involvement of the postsynaptic 5-HT1B site in selectively inhibiting aggression is on the one hand supported by the enhancement of aggressive behavior in 5-HT1B “knockout” mice (Ramboz et al., 1996) but on the other hand is not supported by the anecdotal report that eltoprazine remains effective in decreasing aggressive behavior in these 5-HT1B mutant mice, suggesting involvement of the 5-HT1A receptor (Miczek et al., 1995).

In vivo, alnespirone has a very high and selective affinity ($K_i = 0.19$ nM) for the 5-HT1A receptor site, shows a comparatively low affinity for dopaminergic D2 receptors (20 nM), and exhibits only very low (in the micromolar range) affinity for the 5-HT1B, 5-HT2, alpha and beta adrenergic, D1 dopaminergic, H1 histaminergic, and GABA/benzodiazepine receptor binding sites (Porsolt et al., 1992; Kidd et al., 1993). Given these properties, it already seems most likely that the specific reduction in aggression is mediated through an interaction of this compound at 5-HT1A sites rather than any of the other receptors. Conclusive evidence that the 5-HT1A site is indeed important for the modulation of offensive aggressive behavior comes from the current antagonism studies with the selective 5-HT1A receptor antagonist WAY-100635. Blockade of the 5-HT1A receptors by WAY-100635 not only fully counteracted the effects of alnespirone and the other 5-HT1A agonists but also antagonized the antiaggressive effects of the mixed 5-HT1A/B agonist eltoprazine. This result provides convincing evidence that the 5-HT1A receptor is prominently involved in the antiaggressive effects of these compounds. This is an important observation regarding the aforementioned question of the particular roles the 1A and 1B subtypes play in the modulation of offensive intermale aggression. However, the fact that WAY-100635 was not able to fully block the antiaggressive effect of eltoprazine indicates that the the 5-HT1A Receptor is involved in modulating aggressive behavior as well. Future studies using selective 5-HT1B antagonists should provide conclusive evidence for this involvement. The potent effects of WAY-100635 to reverse the behavioral effects of 5-HT1A agonists agree with its

### Table 1: Effects of different 5-HT1A agonists on behavior in defensive aggression test

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>n</th>
<th>ALT Opponent</th>
<th>Defense</th>
<th>Clinch</th>
<th>Social Explore</th>
<th>Non-social Explore</th>
<th>Groom</th>
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<tr>
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<td>0</td>
<td>38</td>
<td>88.8 ± 22.8</td>
<td>64.2 ± 4.3</td>
<td>9.7 ± 1.0</td>
<td>2.5 ± 1.0</td>
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<td>Alnespirone</td>
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<td>73.9 ± 6.6</td>
<td>8.5 ± 1.2</td>
<td>2.3 ± 1.1</td>
<td>14.3 ± 6.0</td>
<td>0.35 ± 0.35</td>
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<tr>
<td></td>
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<td>12.8 ± 3.7</td>
<td>2.3 ± 1.6</td>
<td>3.9 ± 2.2</td>
<td>0.0 ± 0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>8</td>
<td>69.1 ± 22.6</td>
<td>79.1 ± 5.1</td>
<td>13.4 ± 2.9</td>
<td>1.7 ± 1.4</td>
<td>5.5 ± 3.3</td>
<td>0.0 ± 0</td>
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<tr>
<td>8-OH-DPAT</td>
<td>0.1</td>
<td>8</td>
<td>76.5 ± 47.4</td>
<td>65.0 ± 5.9</td>
<td>15.6 ± 3.7</td>
<td>0.7 ± 0.4</td>
<td>13.5 ± 7.3</td>
<td>1.2 ± 1.2</td>
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<tr>
<td></td>
<td>0.25</td>
<td>6</td>
<td>57.5 ± 29.1</td>
<td>69.1 ± 9.7</td>
<td>10.2 ± 2.9</td>
<td>0.5 ± 0.2</td>
<td>16.9 ± 8.7</td>
<td>2.9 ± 2.8</td>
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<tr>
<td></td>
<td>0.5</td>
<td>8</td>
<td>39.9 ± 11.1</td>
<td>82.5 ± 1.7</td>
<td>8.6 ± 1.9</td>
<td>0.7 ± 0.3</td>
<td>7.3 ± 1.1</td>
<td>0.5 ± 0.4</td>
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<tr>
<td>Buspirone</td>
<td>0.5</td>
<td>7</td>
<td>67.3 ± 40.6</td>
<td>73.8 ± 7.9</td>
<td>12.2 ± 5.3</td>
<td>1.7 ± 0.9</td>
<td>9.5 ± 2.8</td>
<td>0.8 ± 0.6</td>
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<tr>
<td></td>
<td>1.0</td>
<td>7</td>
<td>89.8 ± 51.7</td>
<td>60.9 ± 8.7</td>
<td>15.3 ± 3.5</td>
<td>1.8 ± 1.2</td>
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<tr>
<td>Ispapirone</td>
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<td>8</td>
<td>94.1 ± 27.6</td>
<td>63.7 ± 6.6</td>
<td>9.1 ± 1.9</td>
<td>2.5 ± 1.1</td>
<td>22.8 ± 6.0</td>
<td>1.1 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>8</td>
<td>78.4 ± 29.6</td>
<td>72.4 ± 5.3</td>
<td>12.3 ± 2.2</td>
<td>0.5 ± 0.4</td>
<td>14.3 ± 5.3</td>
<td>0.5 ± 0.3</td>
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<tr>
<td>Eltoprazine</td>
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<td>93.5 ± 31.1</td>
<td>65.3 ± 10.7</td>
<td>6.5 ± 2.1</td>
<td>2.3 ± 1.1</td>
<td>25.9 ± 11.1</td>
<td>0.0 ± 0</td>
</tr>
<tr>
<td></td>
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<td>8</td>
<td>96.4 ± 40.1</td>
<td>64.2 ± 12.8</td>
<td>7.4 ± 1.9</td>
<td>1.1 ± 0.9</td>
<td>27.3 ± 13.2</td>
<td>0.0 ± 0</td>
</tr>
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Data are mean ± S.E.M. ALT, attack latency time (s). Behaviors are expressed as percent of observation time (10 min). ANOVA revealed no significant differences between treatments.
reported selective and efficient antagonistic properties at this site (Fletcher et al., 1996). Recently, it was also reported, similar to our findings, that the antiaggressive effects of 8-OH-DPAT in socially isolated mice were potently blocked by WAY-100635 (Sanchez et al., 1996). Given these previous findings together with our current observations, it seems that the 5-HT₁<sub>A</sub> site is indeed important for the specific modulation of offensive behavior.

Brain 5-HT₁<sub>A</sub> receptors are located postsynaptically on the soma and dendrites of various neurons in the limbic system and cortex (postsynaptic receptors), as well as on the perikarya of serotoninergic neurons in the raphe nuclei (somatodendritic autoreceptors). Activation of the postsynaptic sites results in a reduction of neuronal activity (Andrade et al., 1986; Stevens et al., 1992) and mediates a variety of functional responses, like the induction of flattened body posture and forepaw treading [e.g., symptoms of the 5-HT syndrome: Tricklebank, 1985; Millan et al., 1994; Scott et al., 1994], hypothermia (Millan et al., 1993, 1994), and elevation of plasma adrenocorticotropic hormone (Koenig et al., 1987)]. Activation of the somatodendritic autoreceptors inhibits the firing activity of 5-HT neurons themselves and, consequently, the release of 5-HT from their axonal terminals (Godert et al., 1995) and is thought to underlie the anxiolytic effects of 5-HT₁<sub>A</sub> agonists (Schreiber and De Vry, 1993; De Vry, 1995; Millan et al., 1997). Among the various 5-HT₁<sub>A</sub> agonists, differences exist not only between their potency to stimulate somatodendritic and/or postsynaptic 5-HT₁<sub>A</sub> receptors but also between their ability to activate different subtypes of postsynaptic 5-HT₁<sub>A</sub> sites mediating different functional responses (Millan et al., 1993, 1994; Scott et al., 1994). As expected of its agonist actions at somatodendritic 5-HT₁<sub>A</sub> autoreceptors, alnespirone produced a marked inhibition of the firing of 5-HT neurons and a decrease in 5-HT release and turnover in their projection areas (Kidd et al., 1993; Casanovas et al., 1997), probably underly- ing its anxiolytic-like actions in a variety of behavioral paradigms (Griebel et al., 1992; Persolt et al., 1992; Barrett et al., 1994; Fiele and Andrews, 1994). These somatodendritic 5-HT₁<sub>A</sub> agonist properties of alnespi- rone are similar (albeit with different potencies corresponding to its receptor affinity) to those of 8-OH-DPAT, buspirone, ipsapirone, and eltoprazine (Casanovas et al., 1997). Consistent with its agonist actions at postsynaptic 5-HT₁<sub>A</sub> receptors, alnespirone induces hypothermia (Scott et al., 1994; S. F. de Boer, M. Lesourd, E. Mocaer, and J. M. Koolhaas, unpublished observations) and stimulates the release of adrenocorticotropin and corticosterone (Levy et al., 1995), as has been reported for 8-OH-DPAT, buspirone, ipsapirone, and eltoprazine (Millan et al., 1993; S. F. de Boer, M. Le- sourd, E. Mocaer, and J. M. Koolhaas, unpublished observations). However, in contrast to these other 5-HT₁<sub>A</sub> agonists, alnespirone does not seem to interact with the postsynaptic 5-HT₁<sub>A</sub> receptors that are responsible for inducing the 5-HT behavioral syndrome because alnespirone up to high doses does not cause any of the signs and symptoms of this behav- ior (Scott et al., 1994; Fabre et al., 1997). Furthermore, alnespirone differs from the other 5-HT₁<sub>A</sub> agonists (and/or their metabolites) in that it does not have (ant)agonistic properties at other receptor types in vivo, particularly dopamine D<sub>2</sub> receptors and alpha-2 adrenergic receptors (Van Wijngaarden et al., 1990; Kidd et al., 1993). Although such adrenergic and dopaminergic actions of 5-HT₁<sub>A</sub> ligands were reported to contribute to the unfavorable motor-incapacitating effects seen after 8-OH-DPAT, buspirone, and ipsapirone, our results show that WAY-100635 completely antagonized the inactivity and increase in attack latency induced by all of these compounds. This suggests that the 5-HT₁<sub>A</sub> actions of these compounds are important for these effects. Thus, the combination of very high selectivity for 5-HT₁<sub>A</sub> receptors and differential agonist efficacy at 5-HT₁<sub>A</sub> receptors in postsynaptic target areas of serotonergic projections appears to impart the specific antiaggressive properties of alnespirone.

The next important question to resolve then is whether the specific antiaggressive effects of alnespirone are mediated via its actions at 5-HT₁<sub>A</sub> autoreceptors, thereby inhibiting (stress-activated) global serotonergic neurotransmission and/or via actions at certain postsynaptic 5-HT₁<sub>A</sub> receptors.
located in (fore)brain structures important for modulating offensive aggressive behavior. There are indications in the literature that favor the hypothesis that the antiaggressive effects of 5-HT$1_A$ agonists are exerted via 5-HT$1_A$ autoreceptors in the raphe nuclei to decrease serotonergic activity (see, for example, McMillen et al., 1988), especially the recent observation that the novel benzodioxopiperazine S-15535, which is an agonist and antagonist at presynaptic and postsynaptic 5-HT$1_A$ receptors, respectively, exerts antiaggressive actions (Millan et al., 1997), supports this. Together with findings that the performance of spontaneous or etha-
nol-enhanced aggressive behavior is associated with marked increases in serotonergic activity in selected brain regions (Daruna and Kent, 1976; Garris et al., 1984; Broderick et al., 1984; Haney et al., 1990; Miczek et al., 1994), it is tempting to speculate that activation of somatodendritic 5-HT$1_A$ auto-
receptors, resulting in a decreased (stress/intruder-activated) serotonergic neurotransmission, underlie the specific antiaggressive effects of 5-HT$1_B$ agonists. In conclusion, the present data clearly indicate that al-
nespripine very effectively and specifically suppresses offen-
sive aggression with an advantageous profile of action com-
pared with other full or partial 5-HT$1_A$ agonists. These
selective antiaggressive actions of alnespripine are mediated by stimulat-
ing 5-HT$1_A$ agonists, presumably the somatodendritic autoreceptors at the raphe nuclei. Furthermore, the data also provide evidence for the involvement of these 5-HT$1_A$ receptors in the modulation of aggressive behavior by 8-OH-DPAT, ipsapirone, buspirone, and etoprazine.

Acknowledgments
We gratefully acknowledge the skillful technical assistance of
Ewold ter Veld and Auke Menema in the behavioral observations
and animal care.

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