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Somatodendritic 5-HT_{1A} Autoreceptors Mediate the Anti-Aggressive Actions of 5-HT_{1A} Receptor Agonists in Rats: An Ethopharmacological Study with S-15535, Alnespirone, and WAY-100635

Sietse F. de Boer, Ph.D., Monique Lesourd, Ph.D., Elisabeth Mocaër, Ph.D., and Jaap M. Koolhaas, Ph.D.

To elucidate the relative contribution of somatodendritic 5-HT_{1A} autoreceptors and postsynaptic 5-HT_{1A} receptors in the specific anti-aggressive properties of 5-HT_{1A} receptor agonists, the influence of the novel benzodioxopiperazine compound S-15535, which behaves in vivo *as a competitive antagonist at postsynaptic 5-HT_{1A} receptors and as an agonist at 5-HT_{1A} autoreceptors, upon offensive and defensive aggression was investigated in wild-type rats using a resident-intruder paradigm. S-15535 exerted a potent dose-dependent decrease in offensive, but not defensive, aggressive behavior (inhibitory dose $(ID)_{50}$ = 1.11 mg/kg). This anti-aggressive profile was roughly similar to that of the potent pre- and postsynaptic 5-HT_{1A} full agonist alnespirone (ID_{50} = 1.24). The drug's profound anti-aggressive actions were not accompanied by sedative side effects or signs of the "5-HT_{1A} receptor-mediated behavioral syndrome," which are characteristically induced by prototypical 5-HT_{1A} receptor agonists like 8-OH-DPAT and buspirone. The selective pre- and postsynaptic 5-HT_{1A} antagonist WAY-100635, which was inactive given alone, abolished the anti-aggressive effects of S-15535 and alnespirone, thereby confirming the involvement of 5-HT_{1A} receptors. Furthermore, combined administration of S-15535 and alnespirone elicited an additive anti-aggressive effect, providing further support for somatodendritic 5-HT_{1A} receptor involvement. Finally, the postsynaptic 5-HT_{1A} antagonistic properties of S-15535 were confirmed by showing blockade of the alnespirone-induced hypothermia, a postsynaptic 5-HT_{1A} mediated response in the rat. These data provide extensive evidence that the anti-aggressive effects of 5-HT_{1A} receptor agonists are expressed via their action on somatodendritic 5-HT_{1A} autoreceptors, thereby most likely attenuating intruder-activated serotonergic neurotransmission.*

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KEY WORDS: *Aggression; Resident-intruder; Serotonin; 5-HT_{1A}; Hypothermia; Rat*

A dysregulation of brain serotoninergic (5-HT) transmission, particularly at 5-HT_{1A} receptors, is implicated in the pathophysiology of anxiety, depression, and impulsive states like violent aggression (i.e., Coplan et al. 1995; Maes and Meltzer 1995; Berman et al. 1997). Cor-

From the Department of Animal Physiology (SFB, JMK), University of Groningen, The Netherlands; and the Institut de Recherches Internationales Servier (ML, EM), Courbevoie Cedex, France.

Address correspondence to: Dr. Sietse F. de Boer, Department of Animal Physiology, University of Groningen, PO Box 14, 9750 AA Haren, The Netherlands.

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respondingly, various 5-HT$_{1A}$ receptor agonists exert potent anxiolytic, antidepressant, and anti-aggressive properties in experimental models (see for reviews, De Vry 1995; Broekkamp et al. 1995; Miczek et al. 1995; Olivier et al. 1995) and in man (Stahl 1994; Mann 1995; Ratey et al. 1991).

Brain 5-HT$_{1A}$ receptors are located postsynaptically, on various neurons in the limbic system and cortex, and presynaptically, as somatodendritic autoreceptors on the perikarya of serotoninergic neurons in the dorsal and median raphé (Palacios et al. 1987, 1990). Actions at both pre- and postsynaptic 5-HT$_{1A}$ receptors appears to play a role in the therapeutic properties of 5-HT$_{1A}$ receptor ligands, although their relative contribution remains to be clarified more precisely. To date, the anxiolytic-like effects of 5-HT$_{1A}$ receptor agonists are generally ascribed to a selective stimulation of somatodendritic 5-HT$_{1A}$ autoreceptors, thereby inhibiting the firing activity of 5-HT neurons consequently leading to a transient decrease in (anxiety-enhanced) 5-HT neurotransmission in limbic brain areas (Kidd et al. 1993; Schreiber and De Vry 1993; De Vry 1995; Millan et al. 1997). On the other hand, the rapid (i.e., immediately after drug administration) antidepressant-like effects of the same drugs mainly involve the acute stimulation of certain forebrain populations of postsynaptic 5-HT$_{1A}$ receptors (Martin et al. 1990). Whereas a net increase in the tonic activation of these postsynaptic receptors is thought to underlie the therapeutic effects of several classes of antidepressants for long-term treatment (Blier and de Montigny 1998; Haddjeri et al. 1998).

Concerning the potent anti-aggressive properties of 5-HT$_{1A}$ receptor agonists, evidence on whether this occurs via a pre- or postsynaptic receptor mechanisms is conflicting. (e.g., McMillen et al. 1988; Sijbesma et al. 1991; Mos et al. 1993; Millan et al. 1997; De Almeida and Lucion 1997; Sanchez and Hyttel 1994). However, all these studies investigating the role of pre- and/or postsynaptic receptors in the anti-aggressive actions of 5-HT$_{1A}$ receptor agonists have relied on 5-HT lesion/depletion or intracranial microinjection techniques, and it must be realized that both experimental approaches have limitations. These limitations include changes in aggressiveness due to the 5-HT lesion technique itself, incompleteness of the PCPA and 5,7 DHT induced 5-HT depletions, and spread of the microinjected ligands away from the target sites (Chaput et al. 1990; Jolas et al. 1995).

Recently, a novel pharmacological research tool became available to address this question with the synthesis of the benzodioxopiperazine compound S-15535 (4-(benzodioxan-5-yl)1-(indan-2-yl)piperazine), a highly selective 5-HT$_{1A}$ ligand which behaves *in vivo* as a competitive antagonist at postsynaptic 5-HT$_{1A}$ receptors and as an agonist at 5-HT$_{1A}$ autoreceptors (Millan et al. 1993, 1994, 1997). For example, in line with its agonist action at somatodendritic 5-HT$_{1A}$ autoreceptors in the dorsal raphe nucleus, S-15535 produced a marked inhibition of firing of 5-HT neurons and a decrease in 5-HT release and turnover in their projection areas. Consistent with its antagonist actions at postsynaptic 5-HT$_{1A}$ receptors, S-15535 dose-dependently and completely antagonized postsynaptically mediated responses like spontaneous tail-flicks, flat-body posture, and hypothermia. In accordance with such a unique pharmacological profile are the potent anxiolytic properties in the relative absence of the disruptive motor, autonomic/endocrine and amnesic actions provoked by the activation of postsynaptic sites (Millan et al. 1997).

In order to elucidate the relative contribution of somatodendritic 5-HT$_{1A}$ autoreceptors and postsynaptic 5-HT$_{1A}$ receptors in the specific anti-aggressive properties of 5-HT$_{1A}$ receptor agonists, the present experiments were designed by evaluating the effects of S-15535 on offensive and defensive aggression using a resident-intruder paradigm. For similar reasons, the S-15535 actions were compared with those of alnespirone, a selective full pre- and postsynaptic 5-HT$_{1A}$ receptor agonist, which has a potent and very specific anti-aggressive profile of action (de Boer et al. 1999). Where active, the involvement of 5-HT$_{1A}$ receptors in the actions of S-15535 was confirmed by the use of the novel, highly selective 5-HT$_{1A}$ antagonist, WAY-100635 (Fletcher et al. 1996). Although a WAY-100635-reversible anti-aggressive effect of S-15535 would already strongly indicate a somatodendritic 5-HT$_{1A}$ receptor mediation, putative postsynaptic 5-HT$_{1A}$ receptor involvement was examined by combined treatment of S-15535 and alnespirone. If (part of) the anti-aggressive of alnespirone are postsynaptically mediated, then S-15535 should have an antagonistic action, whereas an additive effect would be expected in the case of a somatodendritic mechanism of action. Finally, the postsynaptic antagonist properties of S-15535 were verified by assessing its ability to prevent the alnepirone-induced hypothermia, which is a postsynaptic 5-HT$_{1A}$ mediated physiological response in the rat (Millan et al. 1993). Some of these results have been published in preliminary form (Mocaër et al. 1996).

MATERIALS AND METHODS

Subjects and Housing

Male Wild-Type Groningen (WTG) rats (*Rattus Norvegicus*; originally wild-trapped animals and bred for approximately 18 generations in our own laboratory under specific-pathogen-free conditions), 4.5 months of age were used as experimental subjects. This strain is preferred for agonistic studies because the rats exhibit an easy to evoke and rich natural repertoire of intraspecific aggressive and social behaviors. They were housed in groups of 5–6 animals from weaning (23 days

after birth) until the start (at age 140 days) of the experiments in clear Plexiglas cages (60 × 60 × 20 cm). The cages were placed in a temperature-controlled room (22 ± 2°C) with a fixed 12 h light/dark photoperiod (lights off at 1300 hr). All aggression tests were performed in the dark-phase between 1400 and 1800 hr. The animals were allowed free access to water and food (Hope Farms Lab chow). All procedures were conducted in conformity with the ethical rules of the Groningen committee on Care and Use of Laboratory Animals.

Behavioral Experimental Procedures

A resident-intruder agonistic paradigm was employed to monitor either offensive behavior (experimental resident) or defensive behavior (experimental intruder) which 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 one week, the baseline level of offensive behavior was tested on three 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 seven animals in clear Plexiglas cages (60 × 60 × 20 cm). Approximately one hour prior to the start of the confrontation, the female partner 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, which showed less than 10% offensive behavior, i.e., ALT > 500 sec, where not included in the drug treatment tests (approximately 15 % of the animals).

On the next day, 20 min before the 10 min confrontation with an intruder, the experimental resident rats received a subcutaneous (s.c.) injection of one of the following test compounds (i.e., animals were tested only once): vehicle (distilled water), S-15535 (0.1, 0.25, 0.5, 1.0, 2.5, 10, or 20 mg/kg), or alnespirone (0.25, 0.5, 1.0, 5.0, or 10 mg/kg). During the 10 min confrontation with an unfamiliar and undrugged conspecific intruder, the full range of behaviors was again recorded. All experimental animals received one drug or vehicle treatment only.

In case of the combination/antagonism studies, vehicle (distilled water) or the putative antagonist compounds were administered 10 min before single challenge doses of the 5-HT_{1A} receptor agonists. Twenty minutes 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-HT_{1A} receptor agonists were based on effective dosages to inhibit aggressive behavior found previously in the dose-response study. The selected dose of WAY 100635 (0.1 mg/kg) was selected on the basis of the results with this compound in previous studies (de Boer et al. 1999).

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. A variety of defensive body postures and escape behaviors of the experimental intruder rat that were recorded accompanied the ensuing agonistic interaction. Therefore, this model gives the opportunity to assess the effects of the 5-HT_{1A} receptor agonists on the complete natural defensive behavioral repertoire. S-15535 (0.5, 1, 2.5, or 10 mg/kg), alnespirone (1, 5, or 10 mg/kg), or vehicle (distilled water) was administered s.c. 20 min before placement of the experimental animal into the home territory of an aggressive male resident for 10 min.

All experimental animals received one drug or vehicle treatment only. The weight of the experimental animals at the time of drug testing ranged from 417 to 493 g. During the 10-min 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, either immediately live or later from the replaying the tapes, manually scored on a data acquisition system. The frequency and duration of salient behavioral elements were recorded in real-time by depressing one of 16 possible keys for the duration of the coded behavior. All behavioral analyses were performed by one person who was blind to the drug treatments. An extensive description of the different behavioral elements displayed during agonistic interactions has been reported previously (Koolhaas et al. 1980, Olivier et al. 1995). Briefly, a total of 28 behavioral elements were scored and grouped into the following behavioral categories to promote a clear representation of the data: 1) *Offense* (lateral threat, clinching, keep down, chasing, upright posture; 2) *Social explore* (moving towards, nosing, investigating opponent, ano-genital sniffing, crawl over, attempted mount, social groom); 3) *Non-social explore* (ambulation, rearing, sniffing, scanning, digging); 4) *Inactivity* (sitting, lying); 5) *Grooming* (washing, shaking, scratching); and 6) *Defense* (submissive posture, keep off, defensive upright, flight, freeze). In addition, the latency time to the first attack, *attack latency time* (ALT) by the resident was taken as a measure of aggressiveness as well. For the present purpose the duration of the different behavioral elements was determined and expressed as a percentage of the total duration of the confrontation.

Biotelemetric Temperature Measurements

In a separate group of WTG animals, body temperature was monitored by means of radio biotelemetry. Transmitters (Model TA10TA-F40; Data Sciences Inc., St. Paul, USA) were implanted intraperitoneally under halothane anaesthesia. After surgery, the animals were housed singly in Plexiglas cages (25 × 25 × 30 cm) on a layer of woodshavings in a room with constant temperature (21 ± 1°C) and fixed 12 h light-dark regime (light on at 0800h). Animals were allowed to recover for at least 10 days, and five days of stable circadian temperature rhythmicity was the prerequisite for the start of the experiments.

The battery-operated transmitter produced temperature dependent output signals (AM frequency in Hz), which were received by an antennae/receiver board (model RA1010, Data Sciences) placed underneath each animal's cage. These units were multiplexed at a consolidation matrix (BCM-100) and connected to a PC-based (IBM Pentium-compatible) data acquisition and analysis system (Dataquest Labpro™, Data Sciences). This system demodulated the signals and converted the raw telemetered data into common units (e.g., °C) using the factory calibration values, and was configured to sample temperature from six experimental animals every 5 min for 10 s on a 24 h basis.

Drug challenge tests were performed between 10 and 11 am during the light cycle. Each animal received all doses of either alnespirone or S-15535 (0, 2.5, 5.0, 10, and 20 mg/kg, s.c.) in a randomized fashion. At least two days separated each challenge test. In the combination study, animals were first injected at t = 0 with either vehicle, 10 mg/kg S-15535 or 20 mg/kg S-15535, and 20 min later with one challenge dose of alnespirone (10 mg/kg).

Drugs

S-15535-3 methanesulfonate (4-(benzodioxan-5-yl)1-(indan-2-yl)piperazine, lot# EI798), molecular weight (mw) 432.5 and alnespirone (S-20499, (S)-N-4-[5-methoxychroman-3-yl)propylamino)butyl-8-azaspiro-(4,5)-diacetamide, hydrochloride); lot# 45109), mw 479 were provided by Institut de Recherches Internationales Servier, France). WAY-100635, mw 513 (lot#A-05;N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N(2-pyridinyl)cyclohexane-carboxamide trihydrochloride), was a generous gift from Wyeth Research (UK) Ltd. All drugs were freshly dissolved in sterile distilled water approximately one hour before the start of the experiments. The injections were given subcutaneously in the flank region in a volume of 1 ml/kg body weight.

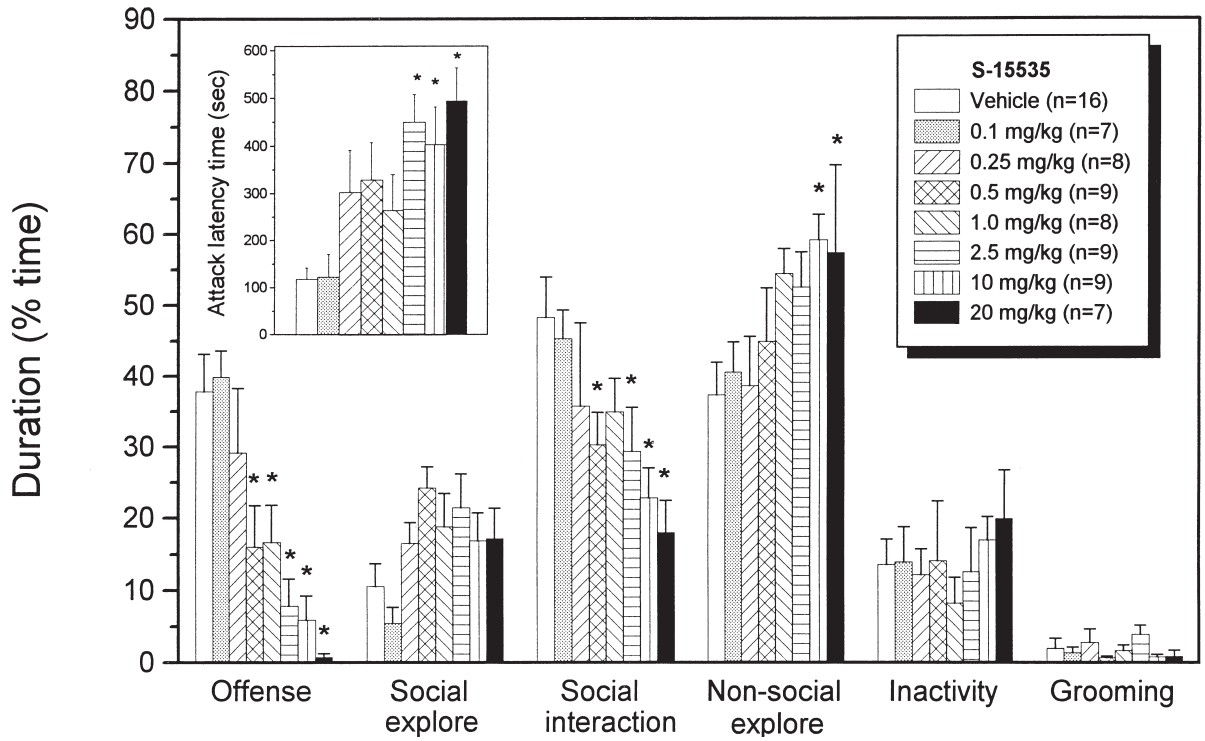

Figure 1. Effect of S-15535 on the attack latency time (ALT; insert) and behavior of resident rats in the offensive aggression test. One-way ANOVA revealed significant effects of drug-dose for ALT [$F(7,65) = 5.28$; $p < .0001$], offensive behavior [$F(7,65) = 7.49$; $p < .0001$], social interaction [$F(7,65) = 6.90$; $p < .0001$], and nonsocial exploration [$F(7,65) = 4.52$; $p < .0001$]. No significant effects of drug-dose were obtained for inactivity [$F(7,65) = 1.37$; N.S.] and grooming [$F(7,65) = 0.53$; N.S.]. * indicates that values are significantly (at least $p < .05$; Dunnett's t-test) different from the vehicle (dose 0) value.

Data Analysis

SPSS 8.0 for Windows 98 was employed to analyze the data statistically. Data are expressed as mean ± standard error (SEM). For most of the variables a Kolmogorov-Smirnov test for normality on the data indicated that the underlying population did not deviate from a normal distribution. Otherwise, the data were square root transformed to normalize, before ANOVA's were performed. For graphical presentation, we did not use these transformed data, but the original mean values, as these are easier to read. The dose-effect curves for each behavioral category 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 category were also computed as percentage of the respective vehicle control values to enable a comparison between various drugs. Least square linear regression analysis was used to estimate the dose (mg/kg) that would elicit 50% aggression reduction (ED_{50}) and the corresponding 95% confidence limits. ED_{50} values with 95% confidence limits that did not overlap were considered to be statistically different. In the antagonist/combination studies, the drug-effect histograms for each behavioral category and attack latency time were analyzed by a two-way ANOVA, pretreatment as between-subject factor one (two levels: vehicle and WAY) and drug as between-subject factor two (two levels).

The temperature responses to the drug injections were assessed for each rat by two parameters: maximum of the change in body temperature after injection as compared to the baseline (mean of the t = −60–t = 0 values; Basal) temperature (MAXdT) and the area under the curve of delta T observed up to 180 min after treatment (AUCdT). These temperature parameters were analyzed using a one-way repeated measure ANOVA. Further analyses were made by Dunnetts' t-tests (between-subject effects) or Student t-tests (within-subject effects) to determine the source of detected significance in the ANOVA's. The criterion of significance was set at $p < .05$.

RESULTS

Offensive Aggression Test: Dose-Response Effects of S-15535 and Alnespirone

Social confrontation initiated by the intrusion of an unfamiliar male rat into the home cage of the territorial ex-

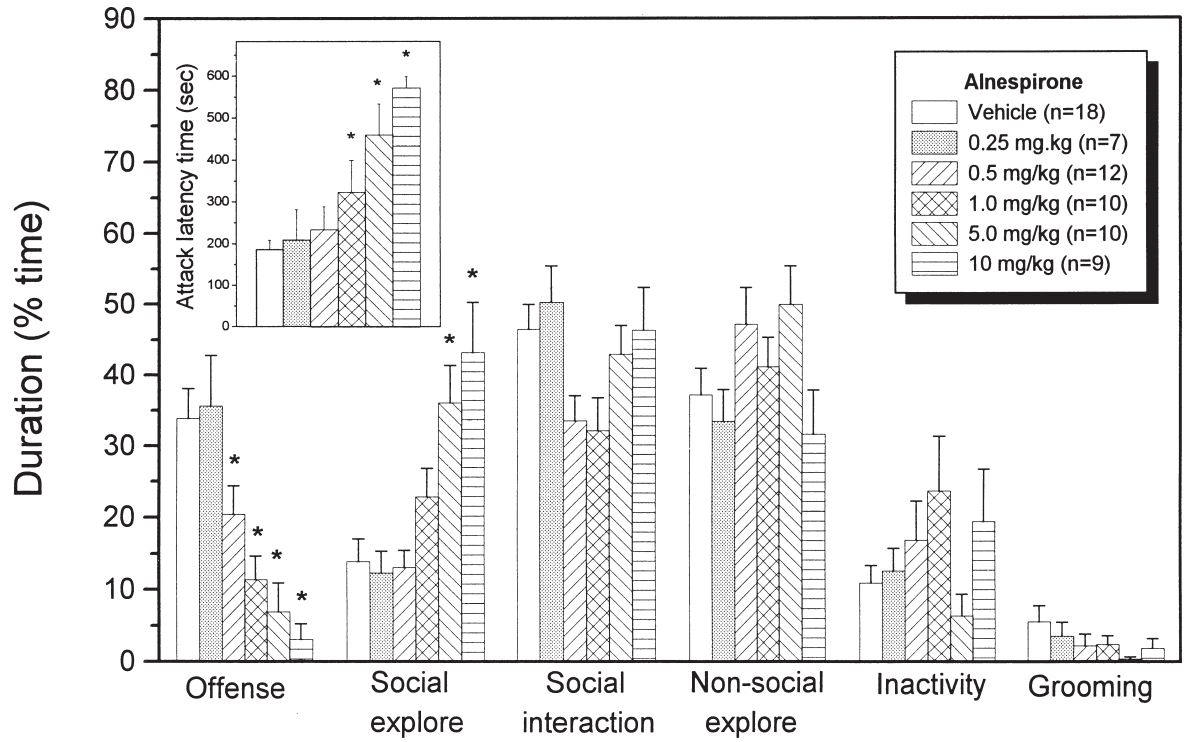

Figure 2. Effect of alnespirone on the attack latency time (ALT; insert) and behavior of resident rats in the offensive aggression test. One-way ANOVA revealed significant effects of dose for ALT [F(5,58) = 7.69; $p < .0001$], offensive behavior [F(5,58) = 10.26; $p < .0001$], and social exploration [F(5,58) = 8.79; $p < .0001$]. No significant effects of drug-dose were obtained for social [F(5,58) = 2.27; N.S.] and nonsocial exploration [F(5,58) = 2.17; N.S.], inactivity [F(5,58) = 0.98; N.S] and grooming [F(5,58) = 1.31; N.S.]. * indicates that values are significantly (at least $p < .05$; Dunnett's t-test) different from the vehicle (dose 0) value.

perimental male counterpart resulted in a typical offensive aggressive behavioral pattern of the vehicle-treated residents, consisting of an approach to 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 vehicle-treated residents (n = 34) ranged from 17 to 280 s with a mean of 118 ± 17 s. These fights always resulted in defeat of the intruder rat which exhibited a variety of defensive/submissive postures and escape responses. Characteristically, several bouts of fighting alternated with periods of no agonistic interactions during the observation trial.

During the 10-min agonistic encounter, vehicle-treated resident rats spent 38.6 ± 4.8% of the time on offensive aggressive behavior, 11.0 ± 2.0% on total social explorative behavior, thus spending 49.6 ± 4.0% on total social interaction. In the remaining part of the 10-min observation period, animals show 34.7 ± 3.9% non-social exploration, 2.0 ± 1.1% grooming, and 13.6 ± 2.7% inactivity (Figure 1). Compared with the vehicle treatment, S-15535-treated rats showed a significant, dose-dependent, delay in the latency time to attack and reduction in the amount of offensive behavior towards the intruder rat. This reduction in offensive behavior was not accompanied by any increase in behavioral inactivity. However, the decrease in offensive behavior was not fully compensated with a concomitant increase in social explorative behavior thus leading to a decrease in total social interaction. In addition, the drug at dosages of 10 and 20 mg/kg did significantly increase non-social explorative activity (Figure 1).

Similar to S-15535 treatment, alnespirone treated rats also showed a pronounced, dose-dependent reduction in offensive aggressive behavior, without affecting behavioral inactivity or grooming. In contrast to S-15535 treatment however, the alnespirone-induced reduction

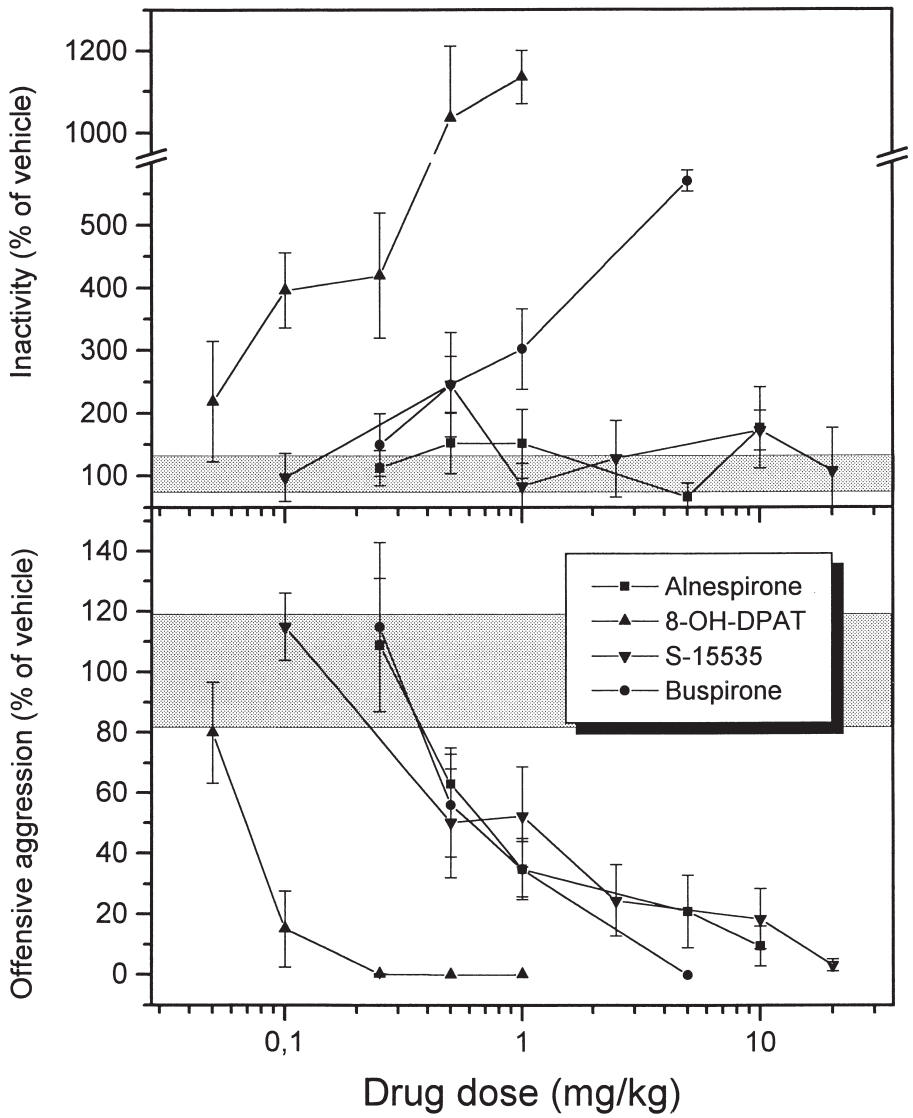

Figure 3. Comparative potency of S-15535, alnespirone, 8-OH-DPAT, and buspirone to inhibit offensive aggression (lower panel) and to enhance behavioral inactivity (upper panel). The anti-aggressive ID_{50} values (95% confidence limits) are for S-15535 1.11 mg/kg (0.79–1.48), alnespirone: 1.24 mg/kg (0.86–1.56), 8-OH-DPAT: 0.074 mg/kg (0.051–0.17), and buspirone: 0.72 mg/kg (0.57–1.06). Data on 8-OH-DPAT and buspirone were taken from a previously published study (de Boer et al. 1999).

in aggression was accompanied by a significant increase in social exploration, thereby leaving total social exploration the same (Figure 2).

Figure 3 shows the comparative potency of S-15535, alnespirone (this study), 8-OH-DPAT, and buspirone (data obtained previously) (de Boer et al. 1999) to inhibit offensive aggression (lower panel) and to enhance behavioral inactivity (upper panel). Clearly, all four ligands exerted a qualitatively similar dose-response pattern to decrease offensive aggressive behavior. The anti-aggressive potency of 8-OH-DPAT (ID_{50} = 0.074 mg/kg) is significantly different from S-15535 (1.11), alnespirone (1.24), and buspirone (0.72), whereas the potencies between S-15535, alnespirone, and buspirone were not significantly different. Figure 3 also more clearly shows the different qualitative and quantitative ability of the four agonists to increase behavioral inactivity: a potent and pronounced dose-dependent increase after 8-OH-DPAT, followed by buspirone, but no increase after any dose of S-15535 or alnespirone.

Antagonism/Combination Studies

Pretreatment with the selective $5HT_{1A}$ antagonist WAY-100635 almost completely prevented the anti-aggressive effects (enhanced ALT, decreased offensive behavior) of S-15535 and alnespirone (Figure 4). The significantly decreased social interaction and enhanced nonsocial exploration observed after treatment with S-15535 were also completely blocked with the WAY-pretreatment, as was the significantly enhanced social exploration induced by alnespirone.

As shown in Figure 5, combined treatment with S-15535 and alnespirone induced an additive anti-aggressive effect.

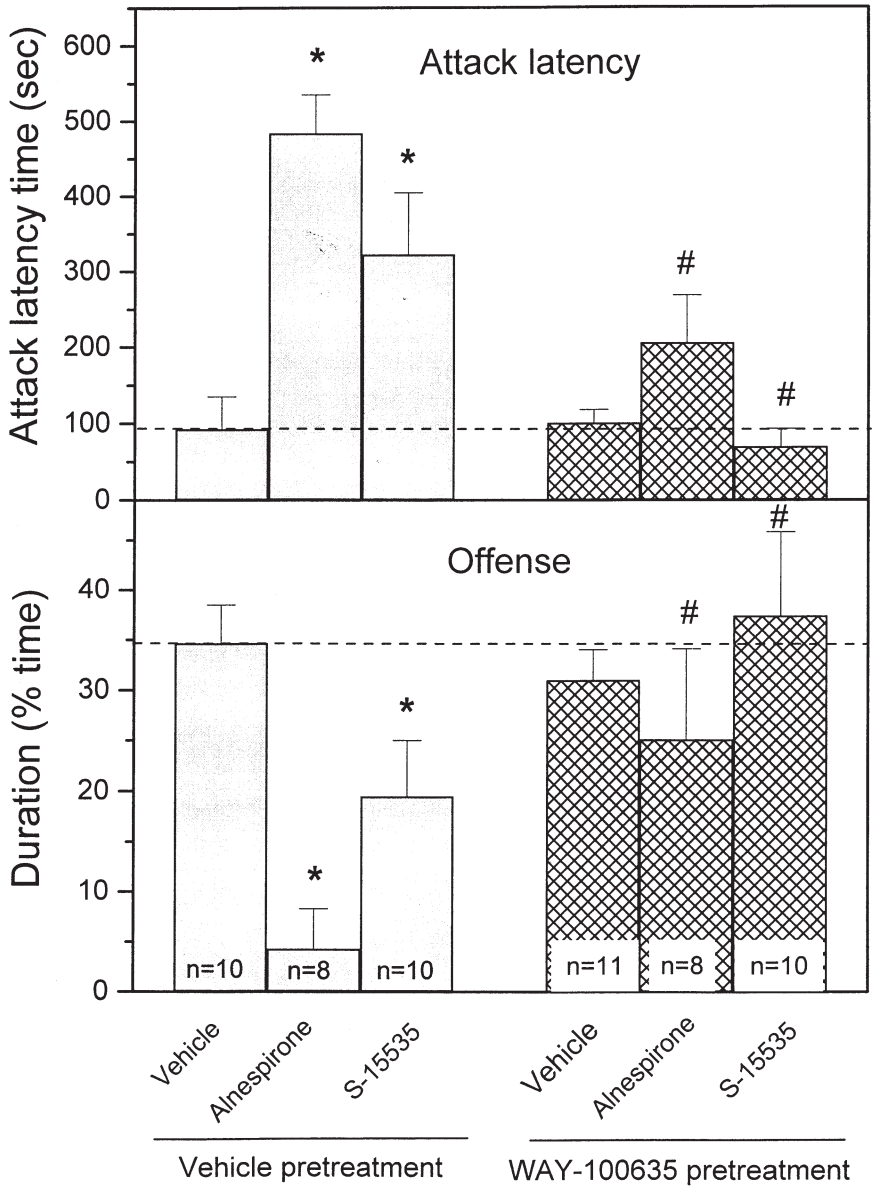

Figure 4. Antagonism of the behavioral effects of S-15535 (2.5 mg/kg) and alnespirone (5 mg/kg) by pretreatment with the $5\text{-}HT_{1A}$ receptor antagonist WAY-100635 (0.1 mg/kg). Data are means ± SEM, n = 8–11 per value as indicated inside the bars. The upper panel shows the effects on attack latency time. Gray-filled bars on the left are vehicle-pretreatments; hatched bars on the right are WAY-100635 pretreatments. Two-way ANOVA revealed significant effects of drug-treatment [$F(2,51) = 15.6$; $p < .0001$], pretreatment [$F(1,51) = 23.4$; $p < .0001$], and drug-treatment × pretreatment interaction [$F(2,51) = 6.9$; $p < .002$]. Bottom panel illustrates the effects on offensive aggressive behavior. Two-way ANOVA revealed significant effects of drug-treatment [$F(2,51) = 7.75$; $p < .001$], pretreatment [$F(1,51) = 22.24$; $p < .001$], and drug-treatment × pretreatment interaction [$F(2,51) = 6.50$; $p < .001$]. * indicate the significance of differences to vehicle/vehicle values; # indicate significance differences to respective vehicle/drug values.

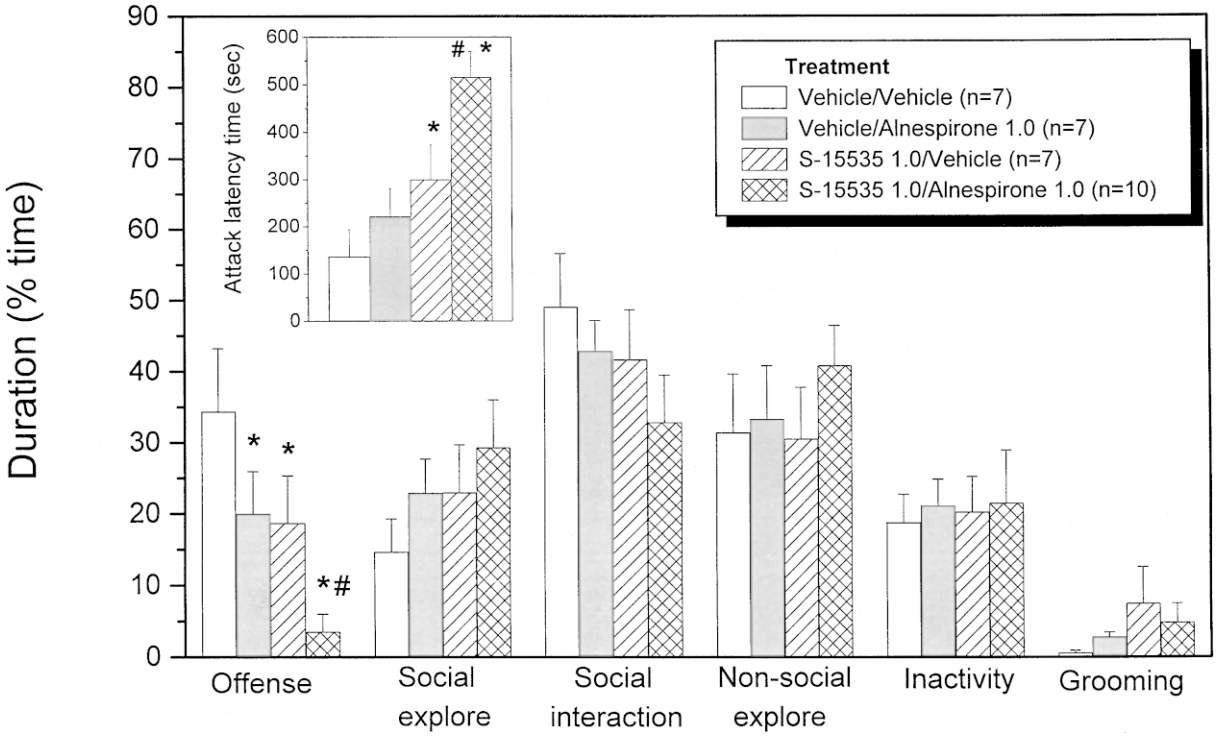

Figure 5. Potentiation of the behavioral effects of alnespirone (1.0 mg/kg) by pretreatment with S-15535 (1.0 mg/kg). Two-way ANOVA revealed significant effects of pretreatment for ALT [F(1,27) = 13.7; $p < .001$] and offensive behavior [F(1,27) = 8.4; $p < .007$], significant effects of the factor treatment for ALT [F(1,27) = 5.9; $p < .02$] and offensive behavior [F(1,27) = 7.1; $p < .01$], and a significant pretreatment × treatment interaction effect for ALT [F (1,27)= 4.32; $p < .05$] and offensive behavior [$F_{(1,27)}$= 4.9; $p < .05$]. * indicates that values are significantly (at least $p < .05$; student's t test) different from the vehicle/vehicle value. # indicate significance differences to respective vehicle/drug values.

This was accompanied by a compensatory increase in social explorative behavior, thereby leaving total social interaction intact.

Defensive Aggression Test

Introduction of a vehicle-treated experimental rat into the home territory of an aggressive trained resident counterpart resulted in an agonistic interaction (mean attack latency time of the resident = 102.7 ± 38 s), leading to a rapid defeat of the experimental intruder. The socially defeated intruder exhibits immediately a variety of defensive body postures and escape behaviors including flight, freezing, defensive upright, keep off, and submission. Vehicle-treated rats spent 60.8 ± 8.2% of the observation time on defensive behavior, 10.9 ± 2.6% on clinching, and the remaining part on social- (4.1 ± 2.7%) and nonsocial exploration (19.9 ± 5.7%).

Within the dose-range tested, S-15535-treated rats showed a similar defensive behavioral pattern when exposed to an aggressive resident as did vehicle-treated rats (Figure 6). In addition, the drug-treated intruders were neither attacked more often than vehicle-treated counterparts, nor was their other behavioral repertoire affected.

Biotelemetric Body Temperature Measurements

As shown in Figure 7 (time-response curves) and Table 1 (deduced parameters), subcutaneous injection of vehicle induced a transient elevation of body temperature, i.e., stress-hyperthermia. Alnespirone treatment (2.5–20 mg/kg) not only prevented this stress-hyperthermia, but also dose-dependently reduced body temperature below pre-injection values, i.e., hypothermia. In contrast, S-15535 (2.5–20 mg/kg) did not induce such a hypothermia but was able to prevent the stress-hyperthermia of vehicle injection. Pretreatment with S-15535 (10 and 20 mg/kg) 20 min before alnespirone injection (10 mg/kg) resulted in a dose-dependent attenuation of the alnespirone-induced hypothermia.

DISCUSSION

The present findings clearly demonstrate that: 1) S-15535, like alnespirone, dose-dependently suppresses offensive aggression without impairment of social, locomotor or defensive behaviors; 2) these specific anti-aggressive effects are effectively blocked by the selective 5-HT$_{1A}$ antagonist WAY-100635, confirming mediation by 5-HT$_{1A}$

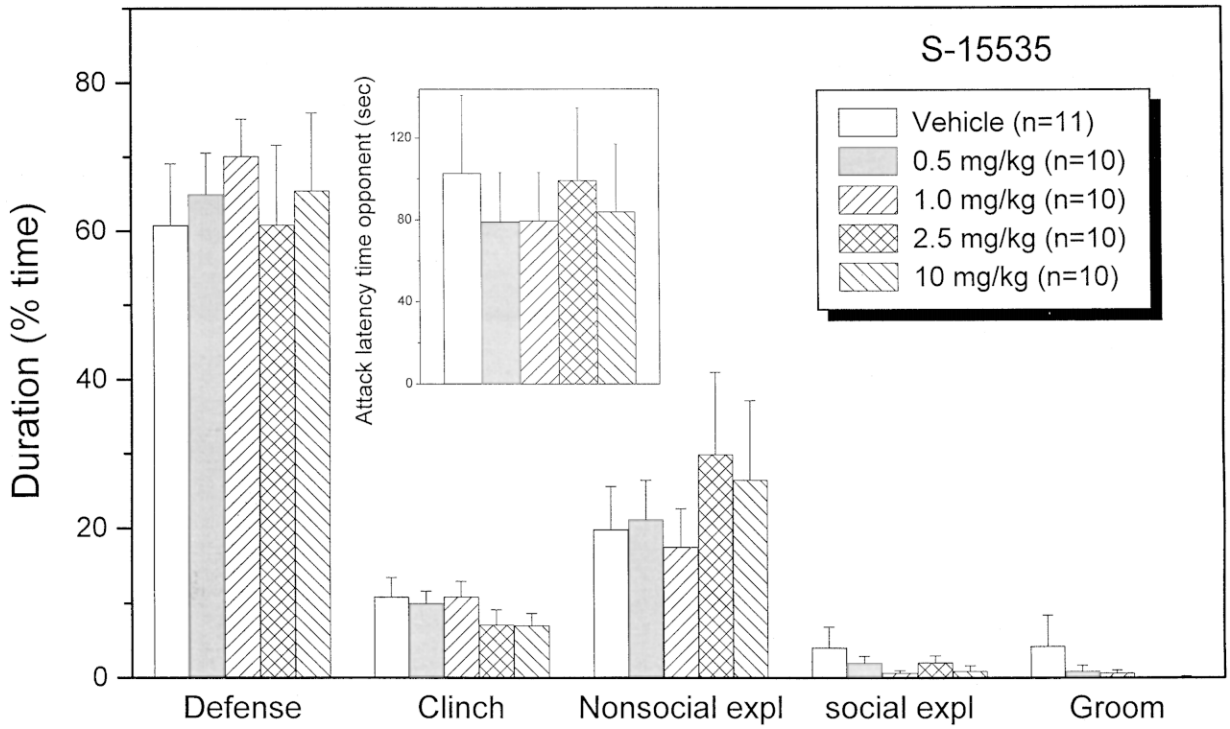

Figure 6. Effect of S-15535 on five different behavioral categories in the defensive aggression test. Insert shows the attack latency time of the resident rats towards the experimental, drug-treated intruder rats. One-way ANOVA yielded no significant main dose effects for any of the behaviors [F(4,46) = <2.6; NS] or resident's ALT [F(4,46) = 0.13; NS].

receptors; 3) combined treatment with S-15535 and alnespirone results in an additive anti-aggressive action, providing further support for somatodendritic 5-HT_{1A} autoreceptor involvement.; and 4) S-15535 attenuated the alnespirone-induced hypothermia, consistent with its postsynaptic 5-HT_{1A} antagonistic properties. Taking into account the rather unique pharmacological profile of S-15535, which acts as an antagonist at postsynaptic 5-HT_{1A} receptors and as a full agonist at 5-HT_{1A} autoreceptors, these data suggest that activation of 5-HT_{1A} autoreceptors mediate the anti-aggressive effects of 5-HT_{1A} ligands.

The potent anti-aggressive properties of 5-HT_{1A} receptor agonists have consistently been reported (Tompkins et al. 1980; Flannelly et al. 1985; Lindgren and Kantak 1987; McMillen et al. 1988; Blanchard et al. 1988; Nikulina 1991; Olivier et al. 1994, 1995; Sanchez and Hyttel 1994; Miczek et al. 1995; Bell and Hobson 1994, Muehlenkamp et al. 1995), but evidence on whether this occurs via a pre- or postsynaptic receptor mechanism is conflicting. Thus, it has been shown that depletion of neuronal 5-HT or lesioning of presynaptic function (with PCPA or 5,7 DHT respectively) either did not change (Sijbesma et al. 1991) or even potentiates (Sanchez and Hyttel 1994) the anti-aggressive effect of 5-HT_{1A} receptor agonists, suggesting a postsynaptic site of action. In line with this are the recently described anti-aggressive effects obtained after local 8-OH-DPAT injection in the dorsal periaqueductal gray and corticomedial amygdala (De Almeida and Lucion 1997). In contrast, however, intraseptal 8-OH-DPAT injection increased aggression (De Almeida and Lucion 1997), whereas local raphe administration of 8-OH-DPAT in low dosages selectively inhibit aggression (Mos et al. 1993; De Almeida and Lucion 1997), indicating a somatodendric mediation of the anti-aggressive effects of 5-HT_{1A} receptor agonists.

Consistent with this are the observations that small doses of nonselective serotonergic antagonists (methysergide), which mainly block postsynaptic receptor sites, do not inhibit but instead potentiate the anti-aggressive activity of 8-OH-DPAT, gepirone, and fluprazine (McMillen et al. 1988). With the exception of this latter study, however, all studies investigating the role of pre- and/or postsynaptic receptors in the anti-aggressive actions of 5-HT_{1A} receptor agonists have relied on lesion and intracranial microinjection techniques, and it must be realized that both experimental approaches have limitations (as mentioned in introduction) (see Chaput et al. 1990; Jolas et al. 1995).

The anti-aggressive profile of S-15535 observed in this study was quite similar to that of the full presynaptic and partial postsynaptic 5-HT_{1A} agonist alnespirone as was recently described (de Boer et al. 1999), i.e., a strong reduction in resident rat's threats, attacks, and pursuits toward an intruder (offensive territorial aggressive elements). These dose-dependent changes in the fi-

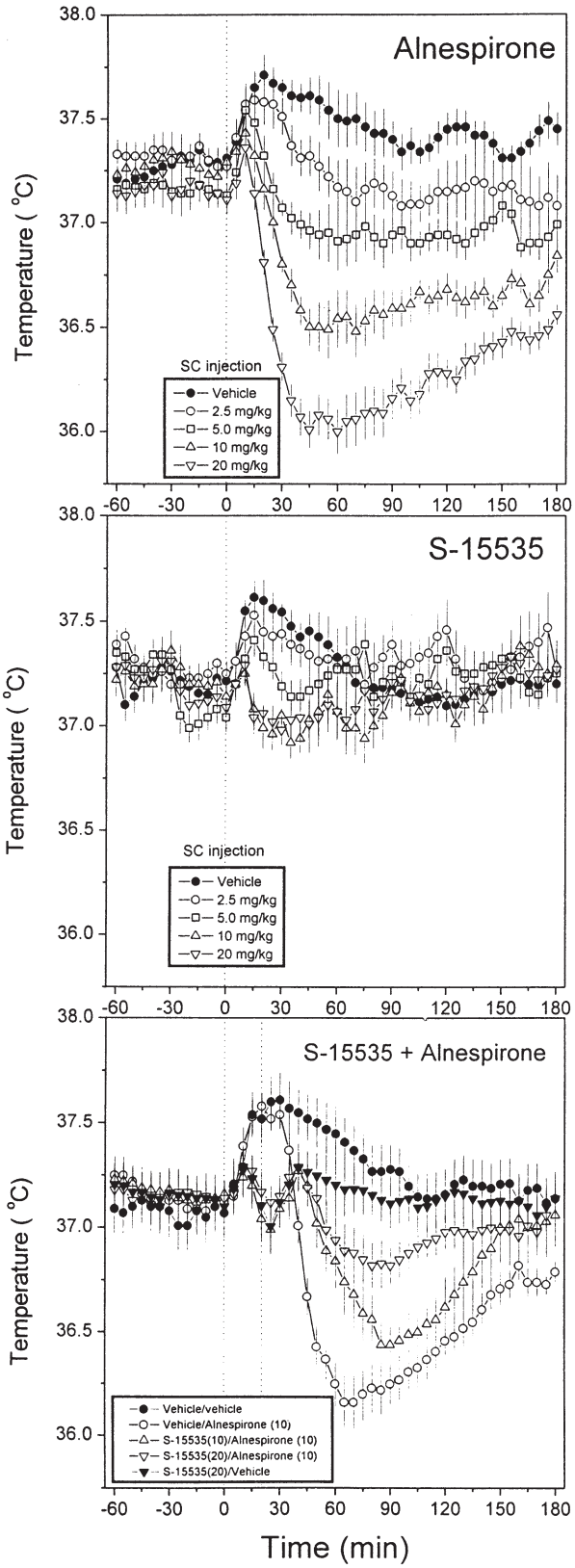

Figure 7. Effects of alnespirone (upper panel), S-15535 (middle panel), and their combination (bottom panel) on body temperature. Temperature was biotelemetrically measured at 5-min intervals for 240 min. At t = 0 min, a s.c. injection of the ligand was given followed, in the case of the combined administration, with a second s.c. injection at t = 20 min. Each data point represents the mean ± SEM of six rats.

nal consummatory parts of the offensive behavioral repertoire are not secondary to any general depressant or motor incapacitating effects of S-15535 because neither behavioral inactivity (sitting, lying, immobility) nor the defensive behavioral repertoire were modified by this drug. Thus S-15535's anti-aggressive effect seems to be very specific in that it is dose-dependent, does not impair normal social interactions and defence/flight abilities, and is without any unwanted motor-effects like general sedation/muscle relaxation. These findings are generally in line with the recent observation that S-15535 inhibited aggression in isolated mice as well (Millan et al. 1997). However, whereas the anti-aggressive effects of alnespirone were accompanied by a compensatory increase in social explorative and contact behavior (thereby not affecting total social interaction), the S-15535 induced anti-aggressive effect was accompanied by an increase in non-social explorative behaviors (rearing, ambulation), thus resulting in a decrease of total social interaction. The reason for this difference in compensatory explorative behavior (social vs. nonsocial) between alnespirone and S-15535 is not clear, but probably not related to the drug's different efficacies at postsynaptic 5-HT$_{1A}$ receptors since S-15535 did not antagonize the alnespirone-enhanced social exploration.

Surprisingly and in contrast to offensive aggression, neither alnespirone (and several other 5-HT$_{1A}$ receptor agonists; i.e., de Boer et al. 1999) nor S-15535 (this study) affected the defensive aggressive behavioral repertoire of experimental intruder rats. Thus, it seems that inhibiting (defeat-enhanced) 5-HT neurotransmission (i.e., net effect of 5-HT$_{1A}$-receptor agonists via activation of the somatodendritic autoreceptors) is not influencing the animals' defence/flight capabilities in reaction to attack by a resident conspecific. From the literature on the neurobiology of aggressive behavior it is well known that different neural substrates are involved in different types of aggressive behavior (Miczek et al. 1994; Olivier et al. 1994). Offensive aggression, characterized by initiative and pro-active behavioral elements, contrasts with the defensive repertoire that is accompanied by submission, flight and more passive/reactive behaviors. It is therefore tempting to hypothesize that the somatodendritic 5-HT$_{1A}$-autoreceptor negative feedback mechanism is particularly involved during situations where animals express proactive behavioral coping responses to a challenge.

The specific anti-aggressive actions of S-15535 and alnespirone are profoundly different from that of other full or partial 5-HT$_{1A}$ receptor agonists like 8-OH-DPAT, buspirone, and ipsapirone which all decrease of-

Table 1. Effect of Alnespirone, S-15535, and Their Combined Treatment on Body-Temperature of Rats

Treatment	Dose (mg/kg)	Basal (°C)	MaxdT (°C)	AUCdT (°C. min)
Alnespirone	0	37.27 ± 0.01	0.44 ± 0.03	35.85 ± 3.42
	2.5	37.33 ± 0.01	−0.25 ± 0.03*	−16.70 ± 3.01*
	5.0	37.15 ± 0.01	−0.31 ± 0.03*	−26.95 ± 2.90*
	10	37.30 ± 0.01	−0.82 ± 0.04*	−91.67 ± 8.67*
	20	37.17 ± 0.02	−1.13 ± 0.06*	−149.00 ± 13.01*
S-15535	0	37.21 ± 0.02	0.42 ± 0.03	23.42 ± 2.43
	2.5	37.23 ± 0.01	0.23 ± 0.04*	10.97 ± 2.56*
	5.0	37.07 ± 0.04	0.14 ± 0.02*	6.62 ± 2.29*
	10	37.24 ± 0.03	−0.05 ± 0.05*	−2.60 ± 2.97*
	20	37.16 ± 0.03	−0.11 ± 0.03*	−7.73 ± 2.78*
S-15535 + alnespirone	0 + 0	37.09 ± 0.02	0.52 ± 0.04	41.15 ± 3.24
	0 + 10	37.10 ± 0.01	−0.99 ± 0.07*	−84.48 ± 4.69*
	10 + 10	37.14 ± 0.01	−0.72 ± 0.08*#	−58.57 ± 4.58*#
	20 + 0	37.16 ± 0.02	0.13 ± 0.02*	−0.34 ± 0.58*
	20 + 10	37.16 ± 0.01	−0.34 ± 0.05*#	−24.02 ± 2.21*#

Values are mean ± SEM of six animals. MAXdT values are the maxium of the change in body temperature after injection as compared to the baseline (mean of the t = 60 − t = 0 values; Basal) temperature. AUCdT values are represented by the area under the curve of delta T observed up to 180 min after treatment).

*Indicate that values are significantly (at least $p < .05$; Dunnett's) different from the respective vehicle (dose 0) value.

#Indicate significant difference from the 20 + 0 condition.

fensive aggression at doses also strongly affecting exploration and motor activity (see Figure 3) (de Boer et al. 1999). These observations of rather unselective anti-aggressive 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; McMillen et al. 1988; Blanchard et al. 1988; Nikulina 1991; Olivier et al. 1994, 1995; Sanchez and Hyttel 1994; Miczek et al. 1995; Bell and Hobson 1994, Muehlenkamp et al. 1995). Actually, the reduction in offensive aggression by these 5-HT_{1A} receptor agonists is generally explained by the predominant quiescent/akinetic (i.e., the 5-HT_{1A} receptor-mediated behavioral syndrome) effects these compounds induce due to their high-efficacy activation of postsynaptic 5-HT_{1A} receptors responsible for this (Tricklebank 1985; ; Scott et al. 1994; Millan et al. 1994).

In contrast to these classical 5-HT_{1A} receptor agonists, alnespirone does not seem to interact with postsynaptic 5-HT_{1A} receptors which are responsible for inducing the 5-HT behavioral syndrome (Kidd et al. 1993; Scott et al. 1994). Indeed, it has been shown that he nature/efficacy of the interaction of alnespirone with a subset of postsynaptic 5-HT_{1A} receptors differs from that of the prototypical agonist 8-OH-DPAT (Fabre et al. 1997). Accordingly, it has been suggested that regional variations in physicochemical or functional properties, i.e., 5-HT_{1A} receptor reserve, receptor-effector coupling (receptor:G-protein stoichiometry), may account, at least in part, for the apparently full 5-HT_{1A} receptor agonist activity of alnespirone and S-15535 in the raphe nucleus, and its partial agonist (alnespirone) or antagonist (S-15535) actions in postsynaptic target areas of serotoninergic projections (Scott et al. 1994; Millan et al. 1994; Newman-Tancredi et al. 1997). Thus, the combination of very high selectivity for 5-HT_{1A} receptors and potent agonist efficacy at the somatodendritic 5-HT_{1A} site appears to impart the specific anti-aggressive properties of alnespirone and S-15535.

Blockade of the 5-HT_{1A} receptors by pretreatment with WAY-100635 completely antagonized the anti-aggressive effects of S-15535 and alnespirone, thereby strongly suggesting the involvement of the 5-HT_{1A} receptors in this action. Given the unique pharmacological profile of S-15535, i.e., antagonist at postsynaptic 5-HT_{1A} receptors and agonist at somatodendritic autoreceptors, these data would provide already enough evidence that the anti-aggressive effects of 5-HT_{1A} receptor agonists are mediated via their action on 5-HT_{1A} autoreceptors. However, to examine the possible involvement of postsynaptic 5-HT_{1A} receptors, a combined treatment of S-15535 and alnespirone was given to check whether the anti-aggressive effect of alnespirone would be antagonized by S-15535. As shown in Figure 5, this antagonism did not occur but instead an additive effect was observed, most likely due to their combined agonist action at the somatodendritic receptor. This result provide additional evidence that activation of somatodendritic 5-HT_{1A} autoreceptors are mediating the anti-aggressive effects of 5-HT1A receptor agonists.

The postsynaptic 5-HT_{1A} antagonistic properties of S-15535 were confirmed by showing a blockade of the alnespirone-induced hypothermia, i.e., a postsynaptic 5-HT_{1A} mediated response (Millan et al. 1993), without inducing a hypothermic response by itself. These re-

sults obtained by biotelemetric temperature measurements replicated the findings of Millan et al. (1993; 1994) using the rectal thermistoprobe procedure. In addition, the alnespirone-induced hypothermia was similar to the reported findings by Scott et al. (1994), and is consistent with the postsynaptic 5-HT$_{1A}$ (partial) agonist property of this drug. Interestingly, S-15535 dose-dependently attenuated the stress-induced hyperthermia, thereby reflecting its reported anxiolytic-like actions (Millan et al. 1997) which are expressed via activation of the somatodendritic receptors. Stress-induced hyperthermia is a common physiological response in rodents when confronted with an aversive/arousing event like a subcutaneous injection (Briese and Cabanac 1991), and therefore also being used as an animal physiological model to study putative anxiolytic-like properties of drugs (Olivier et al. 1994).

Based on our findings it is tempting to hypothesize that 5-HT$_{1A}$-ligand-induced activation of somatodendritic 5-HT$_{1A}$ autoreceptors, resulting in a decreased (intruder-activated) serotonergic neurotransmission, lead to an attenuated display of offensive aggressive behavior. According to this hypothesis, an increased serotonergic activity is linked to the expression of offensive aggressive behavior. Paradoxically however, this view seems to be in apparent contradiction with the well-known "5-HT deficiency" hypothesis of increased aggression (for review see Miczek et al. 1994; Berman et al. 1997), which is almost entirely based on correlative data indicating a long-term trait-characteristic (i.e., low levels of 5-HT metabolism related with a propensity to exhibit heightened aggression or violence).

Our view is based on a short-term dynamic state-characteristic of offensive aggression. Surprisingly, there are virtually no studies that have examined the dynamics of 5-HT neurotransmission during the actual performance of offensive aggressive behavior. There are a number of observations in the literature together with some recent findings from our own laboratory that would favor our hypothesis that the anti-aggressive effects of 5-HT$_{1A}$ receptor agonists are exerted via 5-HT$_{1A}$ autoreceptors in the raphe nuclei to transiently decrease intruder-activated serotonergic activity: 1) several reports indicate that the performance of spontaneous or ethanol-enhanced aggressive behavior is associated with marked increases in serotonergic activity in selected brain regions as estimated by 5HIAA/5HT ratios (Daruna and Kent 1976; Garris et al. 1984; Broderick et al. 1984; Haney et al. 1990; Cadogan et al. 1994), and increased 5-HT neuronal c-fos expression in the raphe nuclei (own unpublished results); and 2) local dorsal raphe administration of eltoprazine and 8-OH-DPAT in low dosages selectively inhibit offensive and maternal aggression (Mos et al. 1993; De Almeida and Lucion 1997). Recent observations (not yet published) in our own laboratory show selective anti-aggressive effects of local dorsal raphe injections of alnespirone and S-15535 as well.

Understanding the exact state of 5-HT neuronal functioning during the initiation and the expression of aggressive behavior is of crucial importance, on the one hand for the compatibility with the 5-HT deficiency hypothesis of enhanced aggression as a trait-characteristic, and on the other hand for effective pharmacotherapeutic interventions of pathological aggressive and violent behavior.

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