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European Journal of Pharmacology 526 (2005) 125–139
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5-HT_{1A} and 5-HT_{1B} receptor agonists and aggression: A pharmacological challenge of the serotonin deficiency hypothesis

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Accepted 23 September 2005

Abstract

More than any other brain neurotransmitter system, the indolamine serotonin (5-HT) has been linked to aggression in a wide and diverse range of species, including humans. The nature of this linkage, however, is not simple and it has proven difficult to unravel the precise role of this amine in the predisposition for and execution of aggressive behavior. The dogmatic view that 5-HT inhibits aggression has dominated both pharmacological research strategies to develop specific and effective novel drug treatments that reduce aggressive behavior and the pharmacological mechanistic interpretation of putative serenic drug effects. Our studies on brain serotonin and aggression in feral wild-type rats using the resident–intruder paradigm have challenged this so-called serotonin deficiency hypothesis of aggressive behavior. The well-known fact that certain 5-$HT_{1A/1B}$ receptor agonists potently and specifically reduce aggressive behavior without motor slowing and sedative effects is only consistent with this hypothesis under the assumption that the agonist mainly acts on the postsynaptic 5-$HT_{1A/1B}$ receptor sites. However, systemic injections of anti-aggressive doses of 5-HT_{1A} and $_{1B}$ agonists robustly decrease brain 5-HT release due to their inhibitory actions at somatodendritic and terminal autoreceptors, respectively. The availability of the novel benzodioxopiperazine compound S-15535, which acts in vivo as a preferential agonist of the somatodendritic 5-HT_{1A} auto-receptor and as an antagonist (weak partial agonist) at postsynaptic 5-HT_{1A} receptors, allows for a pharmacological analysis of the exact site of action of this anti-aggressive effect. It was found that, similar to other prototypical full and partial 5-HT_{1A} and/or 5-HT_{1B} receptor agonists like repinotan, 8-OHDPAT, ipsapirone, buspirone, alnespirone, eltoprazine, CGS-12066B and CP-93129, also S-15535 very effectively reduced offensive aggressive behavior. Unlike the other ligands, however, a remarkable degree of behavioral specificity was observed after treatment with S-15535, in that the anti-aggressive effects were not accompanied by inhibiting (like other 5-HT_{1A} receptor agonist with moderate to high efficacy at postsynaptic 5-HT_{1A} receptors) or enhancing (like agonists with activity at 5-HT_{1B} receptors and alnespirone) non-aggressive motor behaviors (e.g., social exploration, ambulation, rearing, and grooming) beyond the range of undrugged animals with corresponding levels of aggression. The involvement of 5-HT_{1A} and/or 5-HT_{1B} receptors in the anti-aggressive actions of these drugs was convincingly confirmed by showing that the selective 5-HT_{1A} receptor antagonist WAY-100635 and/or the 5-HT_{1B} receptor antagonist GR-127935, while inactive when given alone, effectively attenuated/prevented these actions. Furthermore, combined administration of S-15535 with either alnespirone or CGS-42066B elicited a clear additive effect, indicated by a left-ward shift in their dose–effect curves, providing further support for presynaptic sites of action (i.e., inhibitory somatodendritic 5-HT_{1A} and terminal 5-HT_{1B} autoreceptors). These findings strongly suggest that the specific anti-aggressive effects of 5-HT_{1A} and 5-HT_{1B} receptor agonists are predominantly based on reduction rather than enhancement of 5-HT neurotransmission during the combative social interaction. Apparently, normal display of offensive aggressive behavior is positively related to brief spikes in serotonergic activity, whereas an inverse relationship probably exists between tonic 5-HT activity and abnormal forms of aggression only.

Keywords: Aggression; Resident–intruder; Serotonin; 5-HT_{1A}; 5-HT_{1B}; (Rat)

Contents

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doi:10.1016/j.ejphar.2005.09.065

1. Introduction

A considerable part of our current knowledge on the ethology, neurobiology and, in particular, pharmacology of normal and deviant forms of human aggression is based on experimental laboratory models of aggressive behaviour in animals. Currently, much of this pre-clinical aggression research is conducted in territorial male or maternal female resident rats/mice confronting an intruder conspecific (resident–intruder conflict paradigm). By recording the frequencies, durations, latencies and temporal and sequential patterns of all the observed behavioral acts and postures in the combatants during these confrontations, a detailed quantitative picture (ethogram) of offensive (resident) and defensive (intruder) aggression is obtained (see Olivier and Young, 2004; Miczek et al., 2004; Miczek and de Boer, 2005 for methodological reviews). Using this experimental approach, classical neuroanatomical tracing and electrochemical lesion/stimulation studies have revealed the global neural substrates of aggression (Luiten et al., 1985; Gregg and Siegel, 2001). Several regions of the prefrontal, insular and cingulate cortices, amygdala, septum, medial preoptic area, hypothalamus, periaqueductal gray and their interconnected structures are among the best documented and characterized in this respect. In addition, more recent studies using functional immediate-early gene expression mapping (i.e., c-FOS, p-CREB, zif-268) start to yield a more detailed picture of the individual neurons and their neurochemical identities that become activated within these brain regions during the expression of aggressive behavior (Gammie and Nelson, 2001; Hasen and Gammie, 2005; Kollack-Walker and Newman, 1995; Delville et al., 2000; Halasz et al., 2002; van der Vegt et al., 2003b; Veening et al., 2005—this issue). Furthermore, traditional neuropharmacological studies and new molecular genetic approaches (e.g., gene deletion/overexpression/polymorphism) have resulted in an impressive list of molecular substrates (i.e., neurotransmitters/hormones/cytokines and their respective enzymes, receptors and intraneuronal signalling molecules) that, within this neural circuitry, may profoundly influence resident–intruder aggression (see Nelson and Chiavegatto, 2001; Miczek et al., 2002 for excellent reviews). Despite this expansion, most of these molecules appear to act either directly or indirectly on diverse components of the brain serotonin (5-HT) system. Obviously, this classical neurotransmitter system remains the primary molecular determinant of aggression.

For more than 40 years now, this phylogenetically ancient and anatomically very well conserved indolamine system has been postulated to be essential in the control of aggressive and impulsive behavioral traits in many animal species, ranging from invertebrates like fruit flies, crickets and lobsters (e.g., Kravitz and Huber, 2003) to vertebrates like reptiles (Summers et al., 2003, 2005), fish (Overli et al., 1999; Perreault et al., 2003) birds (Ison et al., 1996), mammals (Miczek et al., 2002) and primates including humans (Tuinier et al., 1995; Berman et al., 1997). Therefore, it should not be surprising that among the most promising pharmacotherapeutic approaches to manage violent and excessively aggressive subjects are those that specifically target 5-HT signaling mechanisms (e.g., activating/inhibiting 5-HT synthesis, release, reuptake, degradation and receptor systems).

Numerous studies over the past two decades have very convincingly shown that pharmacological compounds that activate or antagonize 5-$HT_{1A/IB}$ or 5-$HT_{2A/C}$ receptor subtypes, respectively, potently suppress the display of aggressive behavior in various animal species ranging from invertebrates, fish, rodents, guinea pigs to primates, including man (Tompkins et al., 1980; Olivier, 2001; Benton et al., 1983; Flannelly et al., 1985; Lindgren and Kantak, 1987; Blanchard et al., 1988; McMillen et al., 1988; Parmigiani et al., 1989; Coccaro et al., 1990; White et al., 1991; Sijbesma et al., 1991; Mos et al., 1992, 1993, 1996; Nikulina, 1991; Sanchez et al., 1993, 1996; Sanchez and Hyttel, 1994; Olivier et al., 1989, 1991; Olivier and Mos, 1992; Olivier et al., 1995; Olivier, 2004; Bell and Hobson, 1994; Bonson et al., 1994; Muehlenkamp et al., 1995; Miczek et al., 1989, 1994, 1998, 2002, 2004; Cologer-Clifford et al., 1997; De Almeida and Lucion, 1997; De Almeida et al., 2001; De Almeida and Miczek, 2002; Joppa et al., 1997; Lopez-Mendoza et al., 1998; Simon et al., 1998; Fish et al., 1999; de Boer et al., 1999; Ferris et al., 1999; de Boer et al., 2000; van der Vegt et al., 2001; Buitelaar et al., 2001; Rilke et al., 2001; Sperry et al., 2003; Knyshevski et al., 2005). However, similar to the robust anti-aggressive effects of currently employed pharmacotherapeutic treatments (e.g., anti-psychotics, beta-adrenergic blockers, steroid-derivatives and benzodiazepines), virtually all of these 5-$HT_{1A/1B}$ receptor agonist and 5-$HT_{2A/C}$ receptor antagonist drugs lower aggressive displays in dose

ranges that also severely induce sedation, motor-inactivity, or stereotypies, e.g. rendering them behaviorally nonspecific. This problem of the behavioral specificity (that is, reducing aggressive behavior without concurrently compromising other non-aggressive elements like, sleep, appetite, body-care, sensory and motor activities beyond the normative range) as well as the exact neurobiological mechanism (specific receptor site and location) by which these agents achieve their anti-aggressive effect remain an area of active research.

The behavioral specificity issue can fairly easily be addressed by employing a detailed ethological analyses of all the behavioral elements performed in the offensive aggressive resident–intruder test situation and a comparison of the drug-induced behavioral profile with that of a comparable aggressivity level of drug-free animals. Using such an ethopharmacological approach in either rats or mice, it has recently been claimed that only certain specific 5-HT_{1A} receptor agonists (i.e., alnespirone and S-15535; de Boer et al., 1999, 2000), a mixed 5-$HT_{1A/1B}$ receptor agonist (i.e., eltoprazine; Olivier et al., 1995) and several specific 5-HT_{1B} receptor agonists (i.e., CGS-12066b, CP-94,253, anpirtoline, zolmitriptan, sumatriptan; Bell and Hobson, 1994; Fish et al., 1999; De Almeida et al., 2001; Miczek et al., 2004) exert behavioral specific anti-aggressive effects. In particular, it was claimed that agonists acting on the 5-HT_{1B} receptors have more selective anti-aggressive effects in mice than those acting on 5-HT_{1A} receptors (Miczek et al., 2004; Olivier, 2004). In order to confirm and extend this finding in rats, one goal of this study was to assess and compare the anti-aggressive properties of various 5-HT_{1A} and 5-HT_{1B} receptor selective agonists, with special attention to their behavioral specificity.

Although reversal of the anti-aggressive effects of 5-HT_{1A} and $_{1B}$ receptor agonists by selective 5-HT_{1A} and $_{1B}$ receptor antagonists like WAY-100635 and GR 127935 can confirm the exact receptor type(s) of action (Miczek et al., 1998; Lopez-Mendoza et al., 1998; Fish et al., 1999; de Boer et al., 1999, 2000; De Almeida et al., 2001), the determination of whether this occurs via pre- or post-synaptic sites remains a critical and conflicting issue that is much more difficult to reveal (e.g., McMillen et al., 1988; Sijbesma et al., 1991; Mos et al., 1993; Millan et al., 1997b; De Almeida and Lucion, 1997; Sanchez and Hyttel, 1994). Determination of pre- versus postsynaptic mediation of the anti-aggressive response to 5-HT_{1A} and $_{1B}$ receptor agonists is conceptually important for unraveling the putative inhibitory role of 5-HT neurotransmission in the display of aggressive behavior. This somewhat dogmatic serotonin deficiency hypothesis of aggressive behavior is mainly based on one of the most frequently reported findings in biological psychiatry that excessively aggressive, violent suicidal and impulsive personality traits in human subjects are associated with reduced levels of serotonin's metabolic product 5-HIAA (5-hydroxyindoleacetic acid) in their lumbar cerebrospinal fluid (CSF) and blunted autonomic/neuroendocrine responses to serotonergic challenges (see Berman et al., 1997; Kavoussi et al., 1997 for reviews). Unfortunately and mistakenly, this inverse relationship has been extended toward both species-normative and functionally adaptive levels of aggression in animals, as well as to their actual state-like display of aggressive behavior. Hence, pharmacotherapeutic research strategies have been aimed towards enhancing 5-HT neurotransmission to correct or compensate for the putative hypofunction or deficiency. Moreover, the pharmacological mechanism of serenic drug effects is generally explained by changes in 5-HT neuron function that heighten the effectiveness of serotonergic signalling at their postsynaptic receptor targets. However, in particular the powerful reduction of 5-HT release by 5-HT_{1A} and 5-HT_{1B} receptor agonists at their anti-aggressive dose ranges suggests that the specific inhibition of aggressive behavior is based on a reduction rather than enhancement of 5-HT neurotransmission activity.

It is undisputed that both 5-HT_{1A} receptors and 5-HT_{1B} receptors exist as inhibitory autoreceptors on the serotonergic neurons (5-HT_{1A} on the soma and dendrites in the raphe nuclei; 5-HT_{1B} on the axon-terminals), whereas they are largely inhibitory postsynaptic heteroreceptors in the serotonin system's terminal fields, on target neurons in several corticolimbic regions that govern aggression (Pineyro and Blier, 1999). However, because of this double localization, systemically administered 5-HT_{1A} and 5-HT_{1B} receptor agonists in the anti-aggressive dose-ranges have dual effects on serotoninergic neurotransmission: by acting at the autoreceptors, they very effectively inhibit 5-HT neurotransmission. In contrast, when acting directly at postsynaptic sites, these agonists mimics the effect of 5-HT released, thereby mimicking enhanced 5-HT signalling. Thus, the net effect of a 5-HT_{1A} or 5-HT_{1B} receptor agonist on 5-HT_{1A} and 5-HT_{1B} signal transfer in postsynaptic areas represents a composite of decreased 5-HT release, resulting from autoreceptor activation, and a direct stimulation of the postsynaptic receptors.

To date, virtually all studies investigating whether the anti-aggressive actions of 5-HT_{1A} or 5-HT_{1B} receptor agonists are preferentially mediated by pre- or postsynaptic receptors have relied on either 5-HT lesion/depletion or local intracranial microinjection techniques. It is reasoned that depletion/degeneration of 5-HT-containing neurons via systemic administration of *p*-Chlorophenylalanine (PCPA; irreversible inhibitor of the 5-HT synthesizing enzyme tryptophan hydroxylase) or intracerebral injection of the 5-HT neurotoxic agent 5,7-dihydroxytryptamine (5,7-DHT) would concomitantly remove/decrease somatodendritic 5-HT_{1A} and terminal 5-HT_{1B} autoreceptors. Hence, if the anti-aggressive efficacy of the 5-$HT_{1A/1B}$ and agonists is diminished after these neurotoxic treatments, it would indicate that the presynaptic receptors are involved. If the efficacy is not changed or even augmented, it would indicate that postsynaptic sites are involved. However, it must be realized that both experimental approaches have several pitfalls and limitations (see Discussion section for an elaboration of these). Not surprisingly therefore, the results from these type of studies are rather equivocal.

For the 5-HT_{1A} receptor, a particularly interesting pharmacological research tool became recently available to address this question with the synthesis of the benzodioxane compound S-15535 (4-(benzodioxan-5-yl)1-(indan-2-yl)piperazine), a highly selective 5-HT_{1A} receptor agonist with low intrinsic activity

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, 1994a, 1997a, 2004). 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 5-HT_{1A} responses like spontaneous tail-flicks, flat-body posture and hypothermia (Millan et al., 1994a; Newman-Tancredi et al., 1999; de Boer et al., 2000). 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., 1997b; Cervo et al., 2000). Recently we demonstrated that S-15535 also exerted powerful and selective anti-aggressive effects, already strongly suggesting that a preferential inhibition of ascending serotonergic pathways underlies this serenic effect. To further provide evidence that a transient reduction in serotonergic neurotransmission characterizes the anti-aggressive effects of 5-HT_{1A} and 5-HT_{1B} receptor agonists, this study also investigates the effects of combined administration of S-15535 with either a full postsynaptic 5-HT_{1A}-receptor agonist (alnespirone) or a specific 5-HT_{1B} receptor agonist (CGS-12066B). If (part of) the anti-aggressive effects of alnespirone are postsynaptically mediated, then S-15535 should exert an antagonistic action, whereas an additive effect would be expected in the case of a somatodendritic mechanism of action. Similarly, when (part of) the anti-aggressive effects of CGS-12066B are mediated via activation of postsynaptic 5-HT_{1B} receptors, then S-15535 by inhibiting endogenous 5-HT signaling from these receptor sites should exert an antagonistic action. However, an additive effect could be expected in case of a terminal autoreceptor site of action.

This paper will summarize a set of experiments aimed at additional experimental evidence for the involvement of the somatodendritic 5-HT_{1A} autoreceptor and the terminal 5-HT_{1B} autoreceptor in aggressive behavior, and hence aimed at further challenging the view that serotonin inhibits aggression (i.e., the serotonin deficiency hypothesis of aggression).

2. Materials and methods

2.1. Subjects and housing

Adult male Wild-Type Groningen (WTG) rats (*Rattus norvegicus*; originally wild-trapped animals and bred under conventionalized conditions for 21 generations in our own laboratory, 4.5 months of age were used as experimental subjects). This outbred strain is preferred for agonistic behavior studies because they exhibit an easy to evoke and rich natural repertoire of intra-specific aggressive and social behaviors. Furthermore, their propensity to express offensive aggressive behavior differs widely among individuals from this strain, ranging from no overt aggression at all to very high levels of intense and incessant patterns of aggressive behavior (Fig. 1). They were housed in groups of 5–6 animals from weaning (23 days after birth) until the start (at age 130–140 days) of the experiments in clear Plexiglas cages (60 × 60 × 20 cm). The cages were placed in a temperature-controlled room (21 ± 2 °C) with a fixed 12 h light/dark photoperiod (lights off at 13:00 h). All aggression tests were performed in the dark-phase between 14:00 and 17:00 h. 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 Committee on Care and Use of Laboratory Animals (DEC) of Groningen University.

2.2. Behavioral experimental procedures

Each animal was assessed for the display of aggressive behaviors against an intruder using a standard resident–intruder offensive aggression test (see de Boer et al., 1999 for details). In this test, individual animals are housed in large 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 3 consecutive days during a 10 min confrontation with an

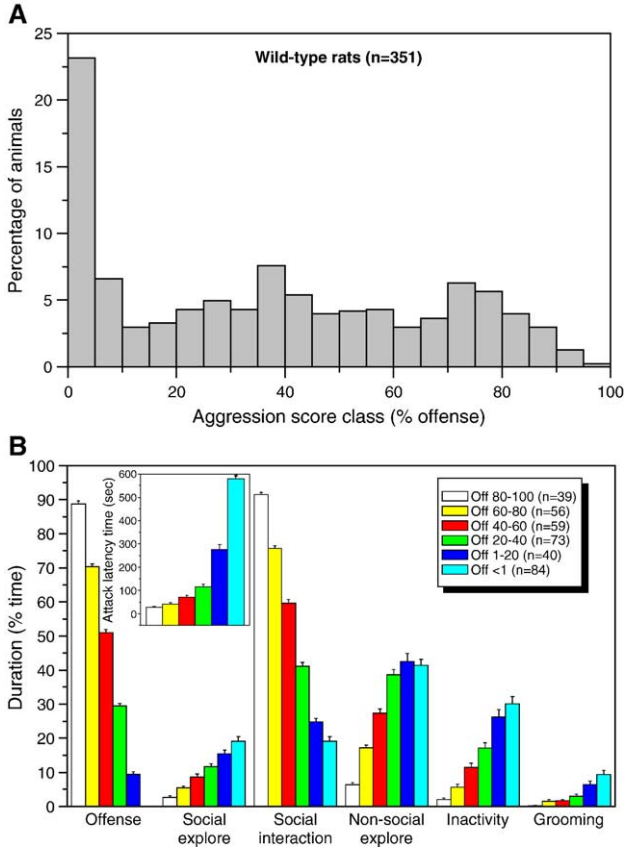

Fig. 1. A: Offensive aggression score distribution of male wild-type rats (n = 351). Data are grouped in classes per 5% time spent in offensive behavior. B: Behavioral profile (percent time spent on the five distinct behavioral categories during a 10 min resident–intruder aggression test) of undrugged wild-type rats used in this study grouped according to their level of displayed offensive aggressive behavior (20% bin classes).

unfamiliar male conspecific (intruder) in the home territory of the experimental (resident) rat. Approximately 60 min prior to the start of the confrontation, the female partner of the experimental resident rat was removed from the observation cage. The naive intruder-rats were socially housed in groups of 7 animals in clear Plexiglas cages (60×60×20 cm). On the fourth day, animals were tested again during which the full range of behavioral elements was recorded (see below). On the next day, 30 min before the 10 min confrontation with an intruder, the experimental resident rats received a s.c. injection of one of the following test compounds: vehicle (distilled water), Repinotan (0.003, 0.01, 0.03 and 0.1 mg/kg), S-15535 (0.063, 0.25, 1, 4 and 16 mg/kg), alnespirone (0.25, 0.5, 1.0, 5.0 or 10 mg/kg), WAY-100635 (0.01, 0.1 and 1.0 mg/kg), eltoprazine (0.1, 0.25, 0.5, 1.0, 2.5 mg/kg), CP-93129 (0.25, 1.0, 4.0, and 16 mg/kg), CGS12066B (0.25, 1.0, 4.0, 16 mg/kg), or GR-127935 (0.25, 1.0, 4.0, 16, mg/kg). In case of the combination/antagonism studies, vehicle (distilled water) or the combination (S-15535, 0.25 mg/kg)/antagonist (WAY-100635, 0.1 mg/kg; GR-127935, 4 mg/kg) compounds were administered 10 min before single challenge doses of the 5-$HT_{1A/1B}$ receptor agonists. Dosages were equally balanced over the animals expressing different levels of aggression. 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, drug combination or vehicle treatment only. The weight of the experimental animals at the time of drug testing ranged from 409 to 495 g.

During the 10-min agonistic confrontations, the full range of behaviors of the experimental resident rat was recorded live and manually scored on a data acquisition system. The frequency and duration of behavioral elements were recorded in real-time by depressing one of 16 possible keys for the duration of the coded behavior. One well-experienced person who was blind to the drug treatments performed all behavioral analyses. An extensive description of the different behavioral elements displayed during agonistic interactions has been reported previously de Boer et al., 2003. 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, immobile, freezing), (5) *Grooming* (washing, shaking,

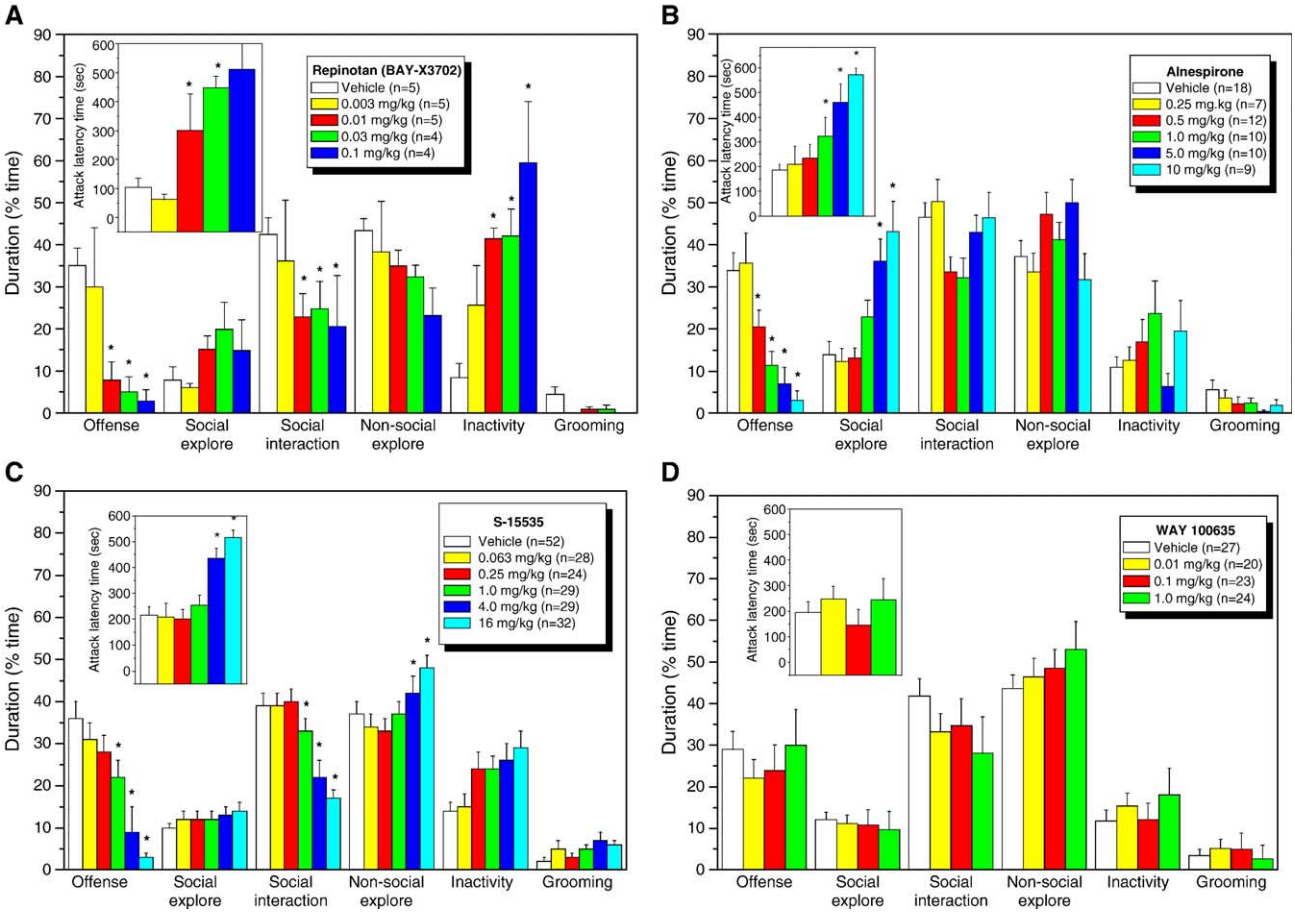

Fig. 2. Effect of different 5-HT_{1A} receptor ligands on behavior of resident rats in the offensive aggression test. A: Repinotan (a selective and highly potent full agonist at both presynaptic and postsynaptic 5-HT_{1A} sites). B: Alnespirone (a selective and potent full agonist at presynaptic and certain postsynaptic 5-HT_{1A} sites). C: S-15535 (a selective and full agonist at presynaptic sites but partial agonist/antagonist at postsynaptic sites). D: WAY-100635 (a selective and potent antagonist at both presynaptic and postsynaptic sites). * Indicates that values are significantly (at least $P<0.05$; Dunnett's *t*-test after ANOVA) different from the vehicle (dose 0) value.

scratching). 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 (see also Fig. 2).

2.3. Drugs

S-15535 (4-(benzodioxan-5-yl)1-(indan-2-yl)piperazine methanesulfonate) and alnespirone (S-20499, (+)-4-[*N*-(5-methoxychroman-3-yl)-*N*-propylamino] butyl-S-azaspiro-(4,5)-decane-7,9 dione) were kindly provided by Drs. Mocaer and Millan from the Institut de Recherches Internationales Servier, France. Repinotan (BAY X-3702, R-(−)-2-{4-[[3,4-dihydro-2H-1benzopyran-2-yl]methyl]anino}butyl)-1,2-benzisothiazol-3(2H)-one 1,1-dioxide monohydrochloride) was generously provided by Bayer AG, Wuppertal, Germany. Eltoprazine dihydrochloride was provided by Solvay Duphar Pharmaceuticals, Weesp, The Netherlands. GR 127935 (*N*-[4-methoxy-3-(4,methyl-1-piperazinyl)phenyl]-2-methyl-4′-(methyl-1,2,4-oxadiozol-3-yl)-[1,1-biphenyl]-4-carboxamide) was donated by Glaxo Wellcome R & D Ltd., Stevenage, UK. WAY-100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-*N*-(2,pyridinyl)cyclohexanecarboxamide trihydrochloride) was obtained from Sigma-RBI. CP-93129 (1,4-Dihydro-3-[1,2,3,6-tetrahydro-4-pyridinyl]-SH-pyrrolo[3,2-b]pyridin-5-one dihydrochloride) and CGS-12066B (7-trifluoremethyl-4-[4-methyl-1 -piperazinyl]-pyrrolo[1,2-a]quinoxaline dimaleate) were obtained from Tocris Cooksen, Ltd., Bristol, UK. Drugs were freshly dissolved in sterile distilled water approximately 1 h before the start of the experiments. The injections (1 ml/kg body weight) were given subcutaneously in the flank region while gently holding the rat on experimenter's lap.

2.4. Data analysis

SPSS 9.0 for Windows 98 was employed to analyze the data statistically. Data are expressed as means of: standard error (S.E.M). For most of the variables a Shapiro–Wilkinson test for normality on the data indicated that the underlying population did deviate from a normal distribution. Therefore, the data were square root transformed to normalize, before Analyses of Variance (ANOVA) were performed. 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 the various drugs. In addition, least square linear regression analysis was used to estimate the dose (mg/kg) that would elicit 50% aggression reduction (ED_{50}) and the

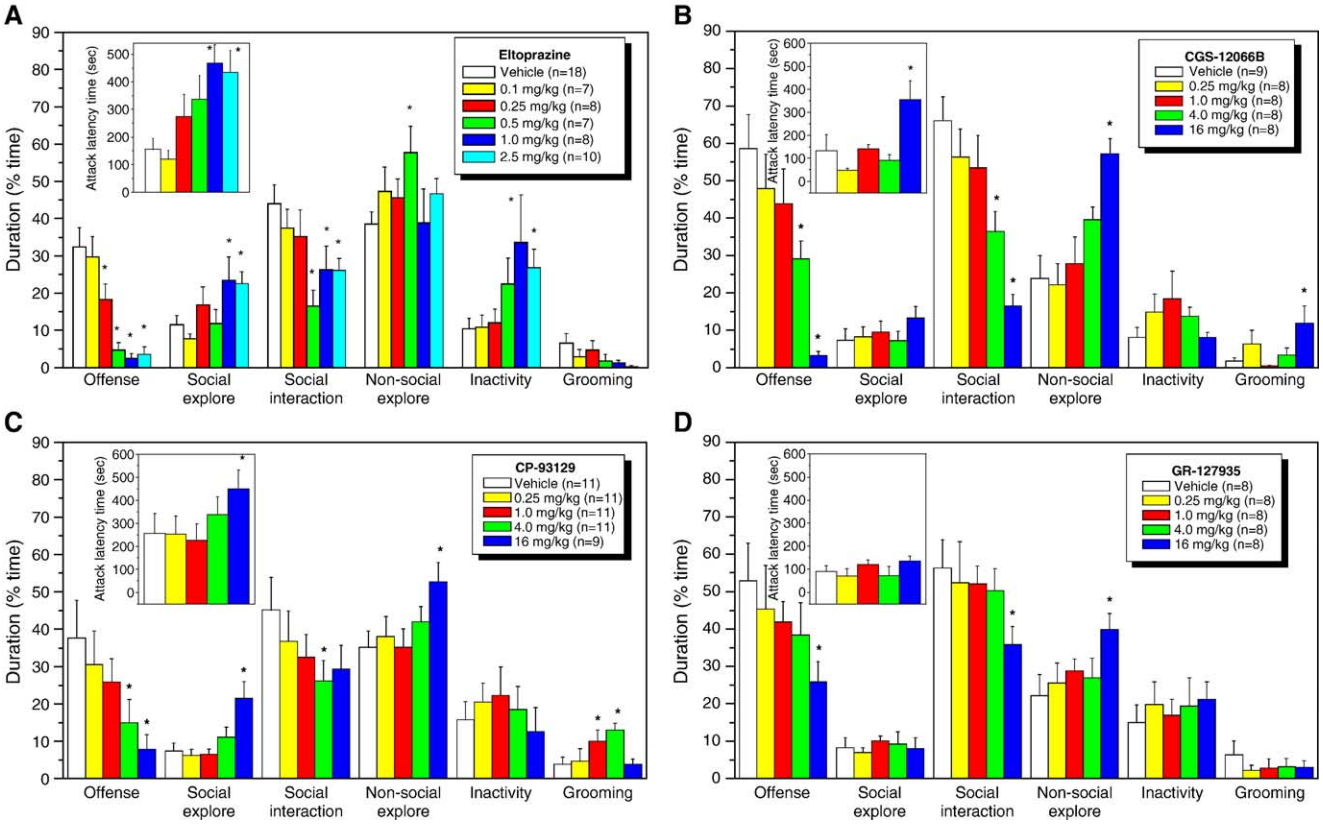

Fig. 3. Effect of different 5-HT_{1B} receptor ligands on behavior of resident rats in the offensive aggression test. A: Eltoprazine (a mixed agonist at both pre- and post-synaptic 5-HT_{1A} and 5-HT_{1B} sites. B: CGS-12066B (a preferential agonist at pre- and post-synaptic S-HT_{IB} sites). C: CP-93129 (a selective agonist at pre- and post-synaptic 5-HT_{IB} sites). D: GR-127935 (a selective and potent antagonist at presynaptic and postsynaptic 5-HT_{1B} sites). * Indicates that values are significantly (at least $P<0.05$; Dunnett's *t*-test after ANOVA) different from the vehicle (dose 0) value.

corresponding 95% confidence limits. ED_{50} values with 95% confidence limits that did not overlap were considered to be statistically different. In the combination/antagonists 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 and drug as between-subject factor two. Further analyses were made by Dunnett's *t*-tests (between-subject effects) or Student *t*-tests (within-subject effects) to determine the source of detected significance in the ANOVAs. The criterion of significance was set at $P<0.05$.

3. Results

3.1. Individual differences in aggression and distributions of aggression scores

As seen previously, individual male resident WTG rats differ widely in their level of species-typical offensive aggression expressed towards an intruder male during the baseline tests, ranging from no overt aggression at all to very high levels of intense and incessant patterns of aggressive behavior. Fig. 1 shows the distribution of all animals used in this study over the various offensive aggression score classes.

Fig. 2 shows the distribution of time spent on five different behavioral categories during the basal (undrugged) offensive aggression test for the total population of 351 animals used in the current studies, grouped according to their level of offensive aggression (bin width 20% offense). Obviously, these groups not only significantly differed from each other as to their level of offensive aggressive behavior but also as to the level of their other four behavioral categories. Thus, the more time animals spent on aggressive behaviors the less time they (can) spent on social and non-social explorative behaviors (i.e., ambulation and rearing), grooming and being inactive. The different behavioral profiles of undrugged animals displaying distinct levels of aggression are relevant for comparison with the behavioral profiles of drug-treated animals that show corresponding levels of aggressive behavior. Interestingly, increased offensive aggression is only partly compensated for by decreased social explorative behaviors. Therefore, the groups of animals can also be clearly differentiated on the basis of their total social interaction time.

3.2. 5-HT$_{1A}$ drug dose–response effects on offensive aggression

Compared with vehicle treatment, all 5-HT_{1A} agonist-treated rats showed a significant, dose-dependent, delay in the latency time to attack and reduction in the duration of offensive behavioral acts and postures towards the intruder rat (Figs. 2 and 4). The anti-aggressive effect of repinotan was associated with a pronounced significant increase of behavioral inactivity and clear signs of the so-called serotonin syndrome of flattened body posture, lower-lip retraction and repetitive motor routines like head-waving and fore-paw treading. In contrast to repinotan, the anti-aggressive effects of alnespirone and S-15535 were not accompanied by increased behavioral inactivity and/or signs of the 5-HT syndrome. Interestingly, the alnespirone-induced reduction in aggression was accompanied by a significant increase in social exploration, thereby leaving total social exploration the same. The behavioral profile of the S-15535 treated animals is virtually similar to the behavioral profile seen in normal undrugged animals performing corresponding levels of spontaneous aggression. Treatment with the 5-HT_{1A} receptor antagonist WAY-100635 had, overall, no effect on the behavioral profile of these rats. At the individual level, however, this drug dose-dependently decreased offense in animals showing high (50%) levels of aggressiveness and increased it in animals with low (<15%) levels of aggressiveness (data not shown).

3.3. 5-HT$_{1B}$ drug dose–response effects on offensive aggression

Compared with vehicle treatment, all 5-HT_{1B} agonist-treated rats showed a significant, dose–dependent reduction in the duration of offensive behavior towards the intruder rat (Figs. 3 and 4). Whereas, similar to the 5-HT_{1A} agonists, eltoprazine also dose-dependently delayed the latency to attack, only the highest doses of CGS-12066B and CP-93129 produced this delaying effect. Although the decreased aggression in eltoprazine-treated animals was associated with increased inactivity, these levels still fell into the normative range when

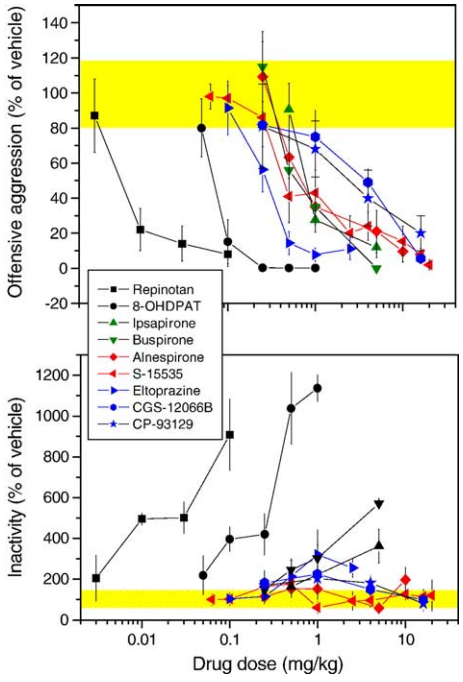

Fig. 4. Comparative potency of different S-HT_{1A} and S-HT_{IB} receptor agonists to inhibit offensive aggression (upper panel) and to enhance behavioral inactivity (lower panel). The anti-aggressive ID_{50} values (95% confidence limits) are for repinotan. 0.0086 mg/kg (0.0041–0.021), 8-OH-DPAT: 0.074 mg/kg (0.051–0.17), ipsapirone: 1.08 mg/kg (0.82–l.55), buspirone: 0.72 mg/kg (0.57–1.06), alnespirone: 1.24 mg/kg (0.86–l.56), S-15535 1.11 mg/kg (0.79–1.48), eltoprazine: 0.24 mg/kg (0.18–0.54), CGS-12066B: 2.31 mg/kg (1.04–2.9) and CP-93129: 1.96 mg/kg (1.01–2.78). Data for and OH-DPAT, ipsapirone and buspirone were taken from a previously published study (de Boer et al., 1999).

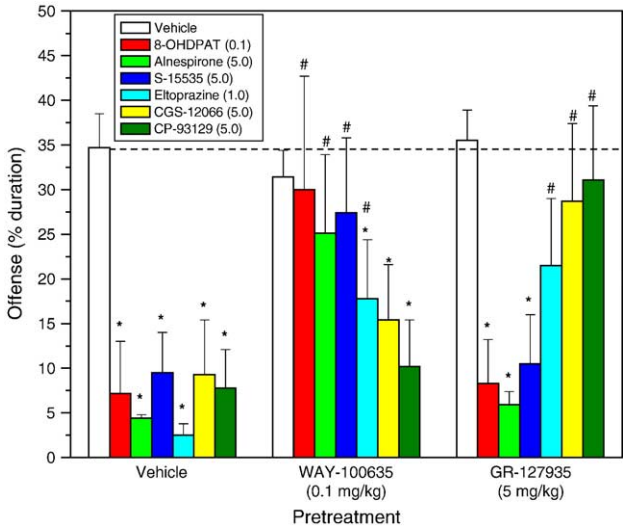

Fig. 5. Effects of pretreatment with the selective 5-HT_{1A} receptor antagonist WAY-10063.5 (0.1 mg/kg) or the 5-HT_{1B} receptor antagonist GR 127935 on the anti-aggressive effects of 8-OHDPAT, alnespirone, S-15535, eltoprazine, CGS-12066B and CP-93129. Data are means±SEM, n=7–12 per value. The horizontal dotted line denotes the control (vehicle/vehicle-treated) level of offensive aggression. Asterisks indicate the significance of differences to vehicle/vehicle and antagonist/vehicle values, # indicate significance differences to the respective vehicle/drug values ($p<0.05$).

compared with the relevant aggression class of undrugged animals. The anti-aggressive effects of CGS-12066B and CP-93129 were accompanied by significantly increased levels of non-social explorative behavior (ambulation and rearing) that, at the highest doses, were also different from the undrugged normative range. Administration of 0.25–4 mg/kg dosages of SR-127935 did not significantly modify the behavioral profile of rats. However, the highest dose (16 mg/kg) significantly attenuated offense and increased locomotor activity.

Fig. 4 (upper panel) shows the comparative potency of several full and partial 5-HT_{1A} agonists (repinotan, S-15535, alnespirone, 8-OH-DPAT, ipsapirone and buspirone), the mixed 5-$HT_{1A/B}$ agonist eltoprazine and the 5-HT_{1B} receptor agonists (CGS 12066B and CP-93129) to inhibit offensive aggression and to enhance behavioral inactivity. Clearly, all tested 5-HT_{1A} and 5-HT_{1B} ligands exerted a qualitatively similar dose–response pattern to decrease offensive aggressive behavior. Quantitatively, however, the anti-aggressive potencies of the full and high efficacy 5-HT_{1A} agonists repinotan (ID_{50}=0.0087 mg/kg) and 8-OH-DPAT (ID_{50}=0.074 mg/kg) are significantly different from the partial and lower efficacious 5-HT_{1A} agonists alnespirone (ID_{50}=1.24), buspirone (ID_{50}=0.72), ipsapirone (ID_{50}=5.5) and S-15535 (ID_{50}=1.11) as well as from the mixed 5-$HT_{1A/B}$ agonist eltoprazine (ID_{50}=0.24) and 5-HT_{1B} agonists CGS-12066B (ID_{50}=2.3) and CP-93129 (ID_{50}=1.96). Interestingly, the potency of the mixed 5-$HT_{1A/B}$ agonist eltoprazine differed from the potencies of the other partial 5-HT_{1A} agonists and from the more selective 5-HT_{1B} agonists. The potencies among these latter ligands were not significantly different. Fig. 4 (lower panel) also more clearly shows the different qualitative and quantitative ability of the 5-HT_{1A} and 5-HT_{1B} agonists to increase behavioral inactivity: a potent and pronounced dose-dependent increase after repinotan and 8-OH-DPAT, followed by buspirone and ipsapirone, but no or only slight (normative compensatory) increases after medium-high doses of eltoprazine, S-15535 alnespirone, CGS-12066B and CP-93129.

3.4. Antagonism/combination studies

Pretreatment with the selective 5HT_{1A} antagonist WAY-100635 (0.1 mg/kg) almost completely abolished the anti-aggressive effects (enhanced ALT, decreased offensive behavior) of 8-OHDPAT, alnespirone and S-15535; only partially blocked the effects of eltoprazine, but did not alter the anti-aggressive effects of CGS-12066B and CP-93129 (Fig. 5). As can be seen in Fig. 6, pretreatment with WAY-100635 (0.1 mg/kg) produced a significant right-ward shift (competitive antagonism effect) in the dose–effect curve of alnespirone's anti-aggressive effects, whereas pretreatment with S-15535 (in a 0.25 mg/kg dose that was ineffective when administered alone) shifted the alnespirone dose–effect curve significantly to the left (additive effect).

Pretreatment with the 5-HT_{1B} antagonist GR-127935 (4 mg/kg) did not alter the anti-aggressive effects of 8-OHDPAT, alnespirone or S-15535, partially reversed the effects of eltoprazine and almost completely blocked the anti-aggressive effects of CGS-12066B and CP-93129 (Fig. 5). In addition, Fig. 7 shows that pretreatment with GR-127935 (4 mg/kg) significantly shifted the anti-aggressive dose–effect curve of CGS-12066B (ID_{50} CGS alone=1.98) to the right (ID_{50} CGS+GR=12.89). Interestingly, pretreatment with the

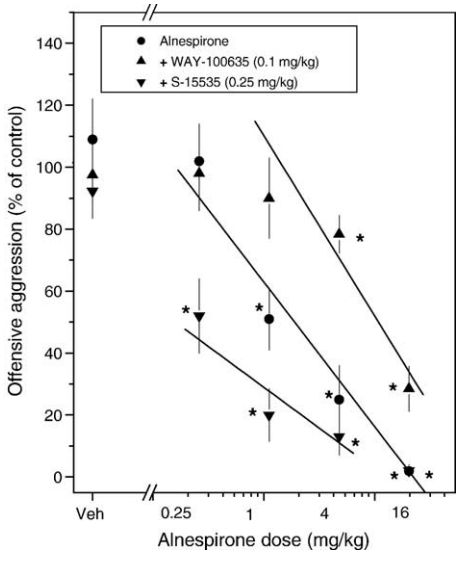

Fig. 6. Potentiation and attenuation of the anti-aggressive effects of 5-HT_{1A} receptor agonist alnespirone by pretreatment with S-15535 (0.25 mg/kg) and WAY-100635 (0.1 mg/kg), respectively. The data from the active, significant portion of the dose range are fit with regression lines to determine the ED_{50} values. * Indicates that values are significantly (at least $p<0.05$) different from the vehicle/vehicle value.

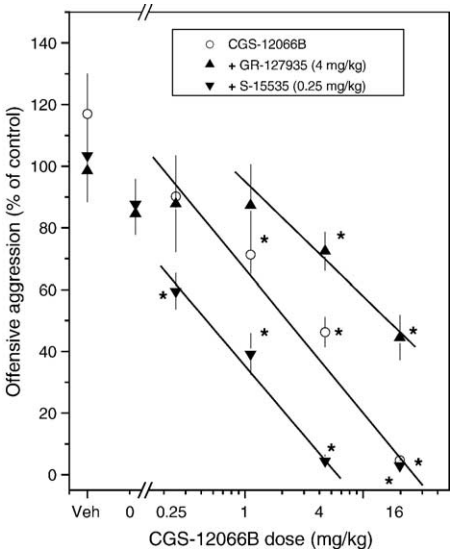

Fig. 7. Potentiation and attenuation of the anti-aggressive effects of the 5-HT_{1B} receptor agonist CGS-12066B by pretreatment with S-15535 (0.25 mg/kg) and GR-127935 (4 mg/kg). The data from the active, significant portion of the dose range are fit with regression lines to determine the ED_{50} values. Asterisks indicates that values are significantly (at least $p<0.0.5$) different from the vehicle/vehicle value.

preferential 5-HT_{1A} autoreceptor agonist S-15535 (0.25 mg/kg) induced a significant left-ward shift in the anti-aggressive dose–effect curve of CGS-12066B (ID_{50} CGS + S-15535 = 0.44).

4. Discussion

The present experiments clearly confirmed that systemic administration of drugs acting as selective agonists on either the 5-HT_{1A} receptor or the 5-HT_{1B} receptor, or on both receptor subtypes, have robust anti-aggressive effects in resident rats confronted with an intruder conspecific. Among all the compounds tested, a remarkable degree of behavioral specificity was observed after treatment with S-15535, in that the anti-aggressive effects were not accompanied by inhibiting (like other 5-HT_{1A} agonist with moderate to high efficacy at postsynaptic 5-HT_{1A} receptors and/or other receptors) or enhancing (like agonists with activity at 5-HT_{1B} receptors and alnespirone) non-aggressive motor behaviors (e.g., social exploration, ambulation, rearing, and grooming) beyond the range of undrugged animals with corresponding levels of aggression. Hence, the anti-aggressive behavioral profile of S-15535-treated animals closely resembles that of undrugged animals displaying comparable levels of offensive aggression.

The reduction of motor activity, at moderate to high doses of the high efficacy 5-HT_{1A} agonists, and the emergence of the so-called serotonin behavioral syndrome (i.e., lower-lip retraction, suppression of rearing, fore-paw treading, flat body-posture, loss of coordination, repetitive motor routines) are clear signs of either their efficacious agonistic properties at postsynaptic 5-HT_{1A} receptors that are known to mediate some of these behavioral incapacitating effects (Hjorth, 1985), or their activity at other receptors (i.e., 5-HT_{1B}, 5-HT2, 5-HT7, α2 adrenoceptors, D2 dopamine receptors). Furthermore, it is well known that activation of postsynaptic 5-HT_{1A} receptor sites induce a wide range of autonomic/endocrine physiological changes (e.g., hypothermia, bradycardia, hypotension, hyperactivity HPA-axis) that most likely also interfere with the proper engagement in and/or expression of aggressive display. This undesirable profile of behavioral physiological effects makes most prototypical 5-HT_{1A} receptor agonists like repinotan, S-OHDPAT, ipsapirone and buspirone unsuitable for clinical use as anti-aggressive drug treatments. In contrast to these classical 5-HT_{1A} receptor agonists, both alnespirone and particularly S-15535 have very distinctive pharmacological profiles of action. Among the various 5-HT_{1A} receptor agonists, differences exist not only between their potency to stimulate somatodendritic and/or postsynaptic 5-HT_{1A} receptors but also between their ability to activate or block different subtypes of postsynaptic 5-HT_{1A} sites mediating different functional responses (Scott et al., 1994; Millan et al., 1994a; Newman-Tancredi et al., 1997). As expected of its agonist actions at somatodendritic 5-HT_{1A} autoreceptors, both alnespirone and S-15535 produce 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 underlying its anxiolytic-like actions in a variety of behavioral paradigms (Porsolt et al., 1992; Griebel et al., 1992; Barrett et al., 1994; File and Andrews, 1994). These somatodendritic 5-HT_{1A} agonist properties of alnespirone and S-15535 are quite similar (albeit with different potencies corresponding to its receptor affinity characteristics) to repinotan, 8-OHDPAT, buspirone, ipsapirone and eltoprazine (Casanovas et al., 1997). Consistent with its agonist actions at selective postsynaptic 5-HT_{1A} receptors, alnespirone induces hypothermia (Scott et al., 1994; de Boer et al., 2000) and stimulates the release of ACTH and corticosterone (Levy et al., 1995; own unpublished findings) as has been reported for repinotan, and OHDPAT, buspirone, ipsapirone and eltoprazine as well (Millan et al., 1993; own unpublished observations). However, in contrast to the other 5-HT_{1A} receptor agonists, alnespirone does not seem to interact with the postsynaptic 5-HT_{1A} receptors that are responsible for inducing the 5-HT behavioral syndrome since alnespirone up to high doses does not cause any of the signs and symptoms of this (Scott et al., 1994; Fabre et al., 1997). Consistent with its antagonistic actions at postsynaptic 5-HT_{1A} receptors, S-15535 dose-dependently and completely blocks postsynaptically mediated behavioral physiological responses like spontaneous tail-flicks, flat-body posture, and hypothermia (Millan et al., 1997b; de Boer et al., 2000). Furthermore, both alnespirone and S-15535 differs from the other 5-HT_{1A} agonists (and/or their metabolites) in that it does not have (ant)agonistic properties at other receptor types in vivo, particularly dopamine D2 receptors and α2 adrenergic receptors (Van Wijngaarden et al., 1990; Kidd et al., 1993; Dugast et al., 1998; Astier et al., 2003). Accordingly, it has been suggested that regional variations in physicochemical or functional 5-HT_{1A} receptor properties, i.e., receptor–reserve or internalization level, receptor–effector coupling efficacy (receptor: G-protein stoichiometry), may account, at least in part, for the apparently full 5-HT_{1A} autoreceptor agonist activity of alnespirone and S-15535 in the raphe nucleus, and its full/partial agonist (alnespirone) or antagonist (S-15535) actions in postsynaptic

target areas of serotoninergic projections (Scott et al., 1994; Millan et al., 1994b; Newman-Tancredi et al., 1997). Thus, the combination of very high selectivity for 5-HT_{1A} receptors, potent agonist efficacy at the somatodendritic 5-HT_{1A} site and lack of agonist activity at postsynaptic 5-HT_{1A} receptors appears to impart the specific anti-aggressive properties of S-15535.

Although it has been claimed that agonists acting on the 5-HT_{1B} receptors have more behaviorally specific anti-aggressive effects than those targeting 5-HT_{1A} receptors (Mos et al., 1993; Olivier, 2004; Fish et al., 1999; Miczek et al., 2002; Miczek et al., 2004), our findings do not entirely confirm this. Compared with most of the full and high-efficiency 5-HT_{1A} agonists, the selective 5-HT_{1B} agonists CGS-12066B and CP-93129 do indeed very effectively suppress aggressive behavior without severely inhibiting other non-aggressive behaviors and thus supporting the claim of more favorable and specific anti-aggressive profiles. Compared with S-15535, treated or undrugged animals, however, it appears that these 5-HT_{1B} receptor agonists promote other non-aggressive motor behaviors as indicated by increases in rearing, ambulation, grooming and/or social exploration. The hyperlocomotor effects of 5-HT_{1B} receptor agonists have been extensively reported before (Green et al., 1984; Goodwin and Green, 1985; Tricklebank et al., 1986; Geyer, 1996; Chaouloff et al., 1999) and this effect appears to be mediated by a presynaptic 5-HT_{1B} heteroreceptor action rather than a 5-HT-release inhibitory effect of the presynaptic 5-HT_{1B} autoreceptors (Green et al., 1984; Oberlander et al., 1987; Tricklebank et al., 1986). Besides hypothermic and neuroendocrine effects, 5-HT_{1B} receptor agonists act on 5-HT_{1B} receptor populations in the endothelium of several vascular beds mediating contractile/dilatory effects resulting in compromised cardiovascular function (Villalon et al., 2003). These physiological effects may prohibit their clinical use as serenic drug.

The involvement of 5-HT_{1A} and/or 5-HT_{1B} receptors in the anti-aggressive actions of the presently tested drugs was convincingly confirmed by showing that the selective 5-HT_{1A} receptor antagonist WAY-100635 and/or the 5-HT_{1B} receptor antagonist GR-127935 effectively attenuated/prevented these actions. Although overall WAY-100635 did not affect the behavioral profiles when given alone, it was noted that it exerted some pro-aggressive effects in animals that showed low undrugged levels of aggressiveness, while it decreased aggression in high-aggressive subjects. Furthermore it was nicely demonstrated that both 5-HT_{1A} and 5-HT_{1B} receptors are involved in mediating the anti-aggressive effects eltoprazine thus confirming its mixed 5-$HT_{1A/1B}$ pharmacological profile of action. Stimulating both 5-HT_{1A} and 5-HT_{1B} (auto)receptors probably also accounts for the higher anti-aggressive efficacy of eltoprazine than the other more selective 5-HT_{1A} and 5-HT_{1B} receptor agonists with comparable intrinsic activities. Indeed, dual 5-HT_{1A} and 5-HT_{1B} activation by combined administration of S-15535 and CGS-12066B produced an anti-aggressive potency (ED_{50}=0.44 mg/kg) quite similar to that of the mixed 5-$HT_{1A/B}$ agonist eltoprazine alone (ED_{50}=0.24 mg/kg). In addition, WAY-100635 tended to attenuate the anti-aggressive effects of CGS-12066B indicating some 5-HT_{1A} agonist properties of this compound. Finally, the fact that the highest dose of the 5-HT_{1B} antagonist GR-127935 attenuated aggression and increased locomotion indicates some intrinsic agonist-like activity of this compound.

After having provided clear evidence of both 5-HT_{1A} and 5-HT_{1B} involvement, the next important issue to address then is whether the anti-aggressive effects of 5-HT_{1A} and 5-HT_{1B} receptor agonists are mediated via its actions at 5-$HT_{1A/1B}$ autoreceptors thereby inhibiting serotonergic neurotransmission, and/or via actions at particular postsynaptic 5-HT_{1A} and presynaptic 5-HT_{1B} heteroreceptors located in (fore)brain regions governing offensive aggressive behavior. To date, virtually all studies investigating whether the anti-aggressive actions of 5-HT_{1A} or 5-HT_{1B} receptor agonists are preferentially mediated by pre- or postsynaptic receptors have relied on either 5-HT lesion/depletion or local intracranial microinjection techniques. With the use of the widely applied former methodology, it is reasoned that depletion/degeneration of 5-HT-containing neurons via systemic administration of PCPA (irreversible inhibitor of the 5-HT synthesizing enzyme tryptophan hydroxylase) or intracerebral injection of the 5-HT neurotoxic agent 5,7-DHT would concomitantly remove/decrease somatodendritic 5-HT_{1A} and terminal 5-HT_{1B} autoreceptors. Hence, if the anti-aggressive efficacies of the 5-$HT_{1A/1B}$ agonists are diminished after these neurotoxic treatments, it would indicate that the presynaptic receptors are involved. If the efficacy is not changed or even augmented, it would indicate that postsynaptic sites are involved. Employing this neurotoxic destruction technique, it was shown that the anti-aggressive effects of 5-$HT_{1A/B}$ receptor agonists remained either unaffected (Miczek et al., 1998; De Almeida et al., 2001) or even increased (Sijbesma et al., 1991; Sanchez and Hyttel, 1994), implicating postsynaptic receptor sites of action. Employing local intracerebral microinjection techniques, it has been shown that intraraphe application of low dosages of 5-HT_{1A} agonists consistently, potently and specifically reduced aggressive behavior (Mos et al., 1993; De Almeida and Lucion, 1997; van der Vegt et al., 2003b) indicating a somatodendritic mediation. More variable, however, local injection of 5-$HT_{1A/B}$ agonists within certain forebrain regions either decreased (Cologer-Clifford et al., 1997; Shaikh et al., 1997; De Almeida and Lucion, 1997; Ferris et al., 1999) or increased (De Almeida and Lucion, 1997) aggression. However, it should be realized that both experimental approaches have several pitfalls and limitations that may render the results from these types of studies rather variable and inconclusive. These problems mainly include: (1) changes in aggressiveness due to the 5-HT lesion technique itself and thereby changes in anti-aggressive drug potency. Usually increases (Soderpalm and Svensson, 1999; Chiavegatto et al., 2001), but also no changes (De Almeida et al., 2001) or even decreases (Sijbesma et al., 1991) in aggression have been reported after depletion of brain 5-HT. (2) Incompleteness and/or variability of the PCPA and 5,7-DHT induced 5-HT neuron destruction, indicating that the contribution of presynaptic sites to the anti-aggressive drug effects cannot be eliminated entirely. In general, 15–40% of the ascending 5-HT neurons are spared after the lesions/depletions as indicated by the remaining tissue

levels of 5-HT and/or density of [3H]paroxetine-labeled 5-HT uptake sites. Furthermore, a recent in vivo microdialysis study has shown that basal levels of extracellular 5-HT are not altered by a near 75% depletion of tissue 5-HT induced by 5,7-DHT injection two weeks before (Hall et al., 1999). (3) Neuronal adaptations due to the depletions that alters the sensitivity of postsynaptic 5-$HT_{1A/B}$ receptors to compensate for the putative reduction in presynaptic sites. Increased sprouting of damaged neurons and supersensitivity of 5-HT_{1A} and 5-HT_{1B} receptors have been demonstrated following neurotoxic lesions (Nelson et al., 1978; Oberlander et al., 1987; Sijbesma et al., 1991; Frankfurt et al., 1994; Van de Kar et al., 1998). (4) Concerning the local intracerebral microinjection technique, spread of microinjected ligands away from the target sites may easily occur (especially with high infusion speed and volumes of solutions) and interferes with the proper interpretation (Jolas et al., 1995). Clearly, while the pre- versus postsynaptic issue of 5-HT_{1A} receptors can be pharmacologically resolved now by use of ligands like S-15535, the role of 5-HT_{1B} auto- and heteroreceptors in the modulation of aggressive behavior remains difficult with the current pharmacological tools. There are no 5-HT_{1B} receptor ligands available yet that may discriminate between presynaptic 5-HT_{1B} auto- and heteroreceptors. Perhaps the application of antisense or RNA-interference methodologies by directly targeting 5-HT_{1B} receptor gene expression in 5-HT and non-5-HT neuron populations could resolve this question in the near future.

There are several observations from both the current and previous studies that favours the hypothesis that the (specific) anti-aggressive effects of 5-$HT_{1A/1B}$ receptor agonists are exerted via 5-$HT_{1A/1B}$ autoreceptors to transiently decrease (social conflict-enhanced) serotonergic activity. (1) Preferential somatodendritic 5-HT_{1A} receptor agonists like BMY-7378 (White et al., 1991), NAN-190 (Sanchez et al., 1996), and in particular S-15535 (Millan et al., 1997a,b; this study) exert potent and selective anti-aggressive actions. (2) Combined administration of S-15535 with either the 5-HT_{1A} receptor agonist alnespirone or the 5-HT_{1B} receptor agonist CGS-12066B elicits a clear additive effect, indicated by a significant left-ward shift in their dose–effect curves (this study). (3) Classical serotonergic antagonists (methysergide), which inhibit postsynaptic receptor sites only, do not block but instead potentiate the anti-aggressive activity of 8-OHDPAT, gepirone and fluprazine (McMillen et al., 1988). (4) Local dorsal raphe administration of 8-OHDPAT, eltoprazine or alnespirone in low dosages selectively into the ventricles inhibit offensive aggression, whereas higher dosages or injections lead to a non-specific reduction of aggression, probably due to spread of the compounds to postsynaptic sites (Mos et al., 1993; van der Vegt et al., 2003b). (5) Administration of 5-$HT_{1A/B}$ receptor agonists within their anti-aggressive dose-ranges invariably suppress 5-HT release (Pineyro and Blier, 1999; Adell et al., 2001). Together, these findings strongly support the idea that the anti-aggressive effects of 5-$HT_{1A/1B}$ receptor agonists are predominantly expressed via their action on 5-$HT_{1A/1B}$ auto-receptors, thereby most likely attenuating a social conflict-activated serotonergic neurotransmission.

Indeed, a number of studies in fish, crustaceans, reptiles and rodents have demonstrated that serotonergic activity in selected brain regions as estimated by 5-HIAA/5-HT ratios is stimulated by the performance of spontaneous or drug-enhanced aggressive behavior (Daruna and Kent, 1976; Garris et al., 1984; Haney et al., 1990; Garris, 2003; Miczek et al., 1994; Huber et al., 1997; Summers and Greenberg, 1995; Summers et al., 2005; Overli et al., 1999, 2001). Moreover, we recently found that the acute and transient display of aggressive behavior (i.e., state-like aggression) was associated with increased 5-HT neural activity as indicated by increased c-*fos* activity in dorsal raphe 5-HT neurons and increased CSF 5-HT turnover (van der Vegt et al., 2003a). Clearly, these findings support an activational role of 5-HT in the initiation and execution of aggressive behavior. The recent pharmacological findings that selective antagonists of postsynaptic 5-HT_{2A} and 5-HT3 receptors exert behavioral specific anti-aggressive effects, further support this view. Finally, the aggression-stimulatory role of 5-HT accommodates well with the major theory on general 5-HT function that activation of the 5-HT system is strongly related to general arousal and motor output (Jacobs and Fornal, 1999; Beekman et al., 2005). Obviously, the serotonin-deficiency hypothesis of aggression is incompatible with this 5-HT hypothesis of facilitating behavioral arousal and motor activity. Serotonergic activity cannot be inhibiting motor patterns of aggression at the same time as it is stimulating motor activity, especially if serotonergic activity is supposed to be uniform (e.g., Summers et al., 2005). Miczek's group (van Erp and Miczek, 2000) tried to clarify this issue using in viva microdialysis in freely behaving (aggressive) rats. They found a clear decrease in the extracellular pool of released 5-HT in the prefrontal cortex but not in the nucleus accumbens shortly after a fight but not before it. No 5-HT changes were measured before or during the initiation and execution of fights. This may be due to the insufficient time resolution of the sampling technique (minutes) relative to the actual behavioral display. The serotonergic system may be characterized by brief spikes of neuronal activity that is associated with certain phases of aggressive intent, posturing and actions, and our current techniques seem to be insufficient to determine the activity of raphe neurons in freely moving animals during these short displays.

The original serotonin deficiency hypothesis was based on a negative correlation between trait-like impulsive aggression/violence and the CSF concentration of the 5-HT metabolite 5-HIAA in humans and other primates (see Berman et al., 1997; Balaban et al., 1996; Kavoussi et al., 1997; Krakowski, 2003 for reviews). Moreover, a substantial number of non-primate animal studies have revealed that the propensity to exhibit excessive and abnormal forms of aggression is similarly linked to long-term reduced brain 5-HT neurotransmission activity (see Miczek et al., 2002 for review). Together with 5-HT manipulation studies demonstrating that chronically lowering or heightening brain 5-HT provokes increased or reduced levels of aggressive behavior, respectively, these studies have strongly supported the serotonergic deficiency hypothesis of trait-like impulsive aggressive behavior. In order to establish and extend this relationship between aggression and 5-HT in our wild-type rats,

a number of studies were carried out. In sharp contrast to the deficiency hypothesis and our expectation, however, a clear positive correlation was found between the level of trait-like aggressiveness and basal CSF concentrations of 5-HT and/or its metabolite 5-HIAA, as well as noradrenaline, dopamine and their metabolites (van der Vegt et al., 2003a). However, tissue as well as microdialysate levels of 5-HT and 5-HIAA in the frontal cortex did not differ significantly between high- and low-aggressive animals, although there was a clear tendency for lower levels in the aggressive animals (de Boer et al., 2003). This negative correlational trend between aggression and frontal cortical 5-HT levels became significant upon inclusion of samples from (abnormal and excessively) aggressive trained fighter animals (unpublished data). A critical evaluation of the 5-HIAA data in aggressive humans revealed that the serotonergic deficiency appears to be most readily detected in circumscribed groups of individuals who engage in impulsive and violent forms of aggressive behavior rather than in individuals with instrumental (functional) forms of aggression (Coccaro, 1989; Mehlman et al., 1994; Tuinier et al., 1995; Balaban et al., 1996). Indeed, normal aggressive behavior aimed at social dominance and social competence is shown to be positively correlated with indices of central and peripheral 5-HT function (Daruna and Kent, 1976; Raleigh et al., 1984). This leads to the hypothesis that the involvement of serotonin in functional aggressive behavior is distinctly different from its role in violence defined as the pathological form of aggression. The evidence presented above suggests that both forms of aggressive behavior are controlled by a rapid but short release of 5-HT during the aggressive act itself. However, in the violent form, this sudden release starts from a strongly inhibited serotonergic tone as a baseline trait characteristic, presumably accompanied/induced by upregulated pre- and/or postsynaptic mechanisms.

We may conclude that the original serotonin deficiency hypothesis may probably hold for the violent, pathological forms of aggressive behavior as a trait only, and that the absolute level of aggressive behavior and its pathological nature is an essential variable in the further analysis of the neurobiology of aggression.

Acknowledgements

The skillful technical assistance of Mrs. Ewold ter Veld and Auke Meinema in the behavioral observations and animal care is gratefully acknowledged.

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