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ORIGINAL INVESTIGATION

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Aggression heightened by alcohol or social instigation in mice: reduction by the 5-HT_{1B} receptor agonist CP-94,253

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Abstract *Rationale:* Models of heightened aggression may be particularly relevant in exploring pharmacological options for the clinical treatment of aggressive and impulsive disorders. *Objectives:* To investigate and compare the effects of a 5-HT_{1B} selective agonist, CP-94,253, on aggression that was heightened as a result of 1) social instigation or 2) alcohol treatment. *Methods:* Male CFW mice were administered 1.0 g/kg EtOH and were subsequently confronted by an intruder in their home cage. In a separate experimental procedure, resident male mice were instigated to aggressive behavior by brief exposure to a provocative stimulus male. To test the hypothesis that activation of the 5-HT_{1B} receptor subtype would preferentially attenuate heightened aggression, in comparison to the moderate levels of species-typical aggressive behaviors, the selective agonist, CP-94,253 (1.0–30 mg/kg, IP), and antagonists to the 5-HT_{1B} (GR 127935; 10 mg/kg, IP) and the 5-HT_{1A} receptor (WAY 100,635; 0.1 mg/kg IP) were used. *Results:* CP-94,253 suppressed non-heightened aggressive behavior (ED_{50}=7.2 mg/kg). GR 127935, but not WAY 100,635 shifted the ED_{50} for CP-94,253 to 14.5 mg/kg. Importantly, the anti-aggressive effects of CP-94,253 were not accompanied by locomotor sedation. Alcohol-heightened and instigation-heightened aggression were suppressed at lower doses than those necessary to suppress non-heightened aggression (ED_{50}=3.8 and 2.7 mg/kg, respectively). *Conclusions:* The current results support the hypothesis that activation of 5-HT_{1B} receptors modulates very high levels of aggressive behavior in a pharmacologically and behaviorally specific manner.

Key words Aggression · Serotonin · Alcohol · Social behavior · 5-HT receptor · Motor activity · Instigation · CP-94,253 · Intruder

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Introduction

Preclinical models of heightened aggression are particularly informative for evaluating pharmacotherapeutic options for the violent patient. Here, we focus on the 5-HT_{1B} receptor as a target. Pharmacological agents directed towards this serotonin receptor subtype may have more behaviorally specific anti-aggressive effects than those of the current treatments, such as antipsychotic medications, β-adrenergic blockers, and selective serotonin reuptake inhibitors (Sbordone and Elliot 1978; Tardiff 1984; Volavka 1995). The effects of potential therapeutic agents on high levels of aggression, in particular, may more accurately predict their efficacy at managing the extreme, pathological forms of human aggression (Eichelman 1992).

Violent individuals are those who engage in intense, and incessant patterns of aggression that fundamentally differ from the adaptive patterns of "instrumental aggression" displayed by various animal species typically used in studies on aggression (Huntingford and Turner 1987; Berkowitz 1993). However, under specific conditions, laboratory animals can be aroused to engage in very high levels of aggression, a pattern of behavior with certain parallels to that of violent individuals. One objective of the current research is to investigate two experimental manipulations, one a pharmacological challenge, the other a social provocation, which engender very high levels of aggressive behavior in mice. We focused on alcohol-heightened aggression because of its paramount public health and social significance (Miczek et al. 1993, 1998a, 1998b). Certain individuals exhibit very large and reliable increases in aggressive behavior under the influ-

ence of a low dose of ethanol, while others are not affected. Secondly, we describe a procedure that triggers escalated aggression by exposure to aggressively arousing or instigating stimuli.

Aggressive arousal has been postulated as an important process for the display of high levels of fighting, ever since Lorenz (1966). In contrast to generalized arousal, this type of aggressive arousal appears to be specific to aggressive behavior, as it is not reflected as increased locomotor, feeding, or sexual behavior (Lagerspetz and Hautojarvi 1967; Potegal and Tenbrink 1984). The social stimuli that precede and occur during an aggressive encounter, such as opponent provocation and reactivity, potently modulate aggressive arousal (Tellegen and Horn 1972; Cairns and Scholz 1973; Thor and Carr 1979; Potegal and Tenbrink 1984; Kudryavtseva 1991). Neurochemically, aggressive arousal seems to be particularly related to the inhibition of brain serotonin (5HT). Levels of both tryptophan and 5-HIAA have been inversely correlated with aggressive behavior in both humans and mice (Valzelli and Garattini 1968; Linnoila et al. 1983; Cleare and Bond 1995). In particular, low CSF concentrations of 5-HIAA have been found in individuals who have a history of impulsive aggressive behavior.

From a pharmacological perspective, research has focused on the 5-HT_1 receptor family as being primarily involved in the suppression of offensive aggression (Lindgren and Kantak 1987; Muehlenkamp et al. 1995). Selective 5-HT_{1A} agonists such as 8-OH-DPAT and flesinoxan attenuate aggressive behavior in mice and rats in a dose-dependent manner (Olivier et al. 1989b; Mos et al. 1993; Sanchez et al. 1993; Miczek et al. 1998b). This anti-aggressive effect can be potently reversed by administering a selective 5-HT_{1A} antagonist such as WAY 100,635 prior to treatment with a 5-HT_{1A} agonist (Miczek et al. 1998b).

The 5-HT_{1B} receptor has become a particular focus of research on aggression ever since the serenic eltoprazine, a mixed 5-$HT_{1A/B}$ agonist, was found to decrease aggression in a relatively specific manner (Olivier et al. 1989a). Further support for the critical role of the 5-HT_{1B} receptor is derived from observing aggressive mutant mice in whom the gene for this receptor has been "knocked out" (Saudou et al. 1994). Recently, a new ligand, CP-94,253, that is selective for the 5-HT_{1B} receptor site has been developed (Koe et al. 1992). Preliminary research with CP-94,253 suggests that it may also be involved in the suppression of aggression which appears to be reversible by pretreatment with GR 127935, introduced as a specific 5-$HT_{1B/D}$ antagonist (Bell et al. 1995; Pauwels 1997).

The present study seeks to investigate the effects of the 5-HT_{1B} agonist CP-94,253 in two models of heightened aggressive behavior, namely alcohol-heightened aggression and instigation-heightened aggression. It is postulated that CP-94,253 should dose-dependently reverse both the pharmacological and behavioral enhancement of aggression that is observed in these models. In addition, this effect is believed to be specific for the 5-HT_{1B} receptor such that the reversal of this phenomenon would occur under pretreatment with the 5-$HT_{1B/D}$ antagonist, GR 127935, but not with the 5-HT_{1A} antagonist, WAY 100,635.

Materials and methods

Subjects

Adult male CFW mice (Charles River Breeding Labs, Wilmington, Mass., USA), arrived at 5 weeks old, weighing 20–25 g. As defined below, some of these mice were designated as "residents" (n=69). Each resident male was housed with a female of the same strain in 28×17×14 cm clear, polycarbonate, cages. The cages were covered by a stainless steel, wire lid. Wood shavings were on the floor and were changed once a week. Rodent chow (Purina) and tap water were freely available through the cage lid. Pups born to these pairs were weaned at three weeks of age. Additional male CFW mice (n=276) were referred to as "intruders" and housed in groups of four to six. The vivarium was maintained at 21±1°C, humidity 30–40%, and a 12-h light/dark photocycle (lights on at 0800 hours). The mice were cared for according to the guidelines of the "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985). All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Tufts University.

Apparatus and measurements

The behavior of the mice was recorded by a low lux video camera connected to a standard video cassette recorder. The behaviors were analyzed by trained observers using a custom-designed data acquisition program, similar to that previously described (Miczek 1982). The frequency and duration of each operationally defined behavior were coded by depressing a specific key on a special keyboard. Aggressive and non-aggressive behaviors were defined according to the descriptions of Grant and MacKintosh (1963) and as illustrated by Miczek and O'Donnell (1978). The salient aggressive behaviors comprised: anogenital contact with the intruder, pursuit, sideways threat, bite, and tail rattle. Non-aggressive behaviors included: grooming, walking, and rearing. Inter- and intra- observer reliability was calculated using the Spearman correlation coefficient and ranged from 0.92 for the duration of walking to 0.98 for the frequency of attack bites.

Drugs

Ethyl alcohol (100%, AAPER Alcohol, Shelbyville, Ken., USA) was diluted to 10% (w/v) with distilled water and injected orally in a volume of 1 ml/100 g body weight. CP-94,253 (3-(1,2,5,6-tetrahydro-4-pyridyl)-5-propoxypirolo[3,2-b]pyridine) (Charles Pfizer, Groton, Conn., USA) was dissolved with the aid of sonication in a vehicle of 5% Tween 80, 5% DMSO (dimethyl sulfoxide) and 90% distilled water. GR 127935 (*N*-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxadiozol-3-yl)-[1,1-biphenyl]-4-carboxamide) (Glaxo Research and Development Ltd) was dissolved in 10% hydroxypropyl-betacyclodextrin in dH_2O. WAY 100,635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl) cyclohexancarboxaminde trihydrochloride; American Home Products, Ayerst Wyeth) was dissolved in 0.9% saline. All drugs except ethanol were administered IP in a volume of 1 ml/ 100 g body weight.

Experimental procedures

Resident-intruder confrontations

Initially, each resident was assessed for the display of aggressive behaviors against an intruder. After the removal of the female and any pups, a group-housed male mouse was placed into the home cage of the residents for 5 min (Miczek and O'Donnell 1978). Once the residents attacked the intruders reliably, typically after three to four confrontations, they were adapted to either an oral or IP injection administered 15 min prior to the encounter with the intruder. During the adaptation phase, the aggressive behavior of the mice became less variable than during the first confrontations (Winslow and Miczek 1984). Experimental sessions were recorded from the introduction of the intruder to the termination of the test, 5 min after the first attack bite. If no attack bite occurred, the experimental session was terminated at 5 min. The frequencies and durations of the salient agonistic behaviors were analyzed at a later time. Experimental sessions were scheduled between 0900 and 1900 hours at 3- to 4-day intervals.

CP-94,253 dose effect and antagonism

Fifteen animals were used as subjects in the CP-94,253 dose-effect study. CP-94,253 or its vehicle (5% Tween 80, 5% DMSO in dH_2O) was administered to the resident IP 30 min prior to the introduction of the intruder. Subjects received every dose of CP-94,253 (3.0, 5.6, 10.0, 17.0 mg/kg) in a counterbalanced sequence. The dose range and timing of drug injection were similar to that used by Lee and Simansky (1997) investigating the effects of CP-94,253 on feeding behavior. One drug test and one vehicle test were performed each week.

In another study, mice were pretreated with a 0.1 mg/kg dose of WAY 100,635 before the administration of CP-94,253 (n=13). WAY 100,635 was given IP 15 min prior to injection of CP-94,253 (0, 5.6, 10.0, 17.0 mg/kg) (Miczek et al 1998a). The sequence of doses was counterbalanced, one drug test and one vehicle treatment per week.

Subsequently, an antagonism study with GR 127935 was performed (n=11). All mice were pretreated with a 10.0 mg/kg dose of GR 127935. GR 127935 was administered IP 30 min prior to injection of CP-94,253 (0, 10, 17, 30 mg/kg) (Parsons et al. 1998). The sequence of drug doses was counterbalanced, and every drug test was preceded by a vehicle test.

In all three studies, if a carry-over effect from the previous drug test was apparent, as indicated by a change from baseline >20%, the vehicle test was repeated until the attack rates returned to baseline levels.

Alcohol-heightened aggression

In a new set of resident mice (n=41), individuals were identified who demonstrated very large increases in aggressive behavior in response to a challenge with the maximally effective dose of ethanol (1.0 g/kg, PO) (Miczek et al. 1993, 1998a, 1998b). The data from two ethanol challenges were compared to those from five vehicle control tests that preceded and followed the ethanol test days. Multiple vehicle control tests were performed in order to ascertain the stable baseline level of aggression. Based on these comparisons, the mice were assigned to the following subgroups, as previously described (Miczek et al. 1998a, 1998b): (1) alcohol-heightened aggression (AHA), n=10 and (2) alcohol-non-heightened aggression (ANA), n=31. For each individual mouse, the average frequency of attacks during the ethanol tests was compared to the average frequency of attacks during the vehicle control tests. If the alcohol tests exceeded the individuals' vehicle control mean by 2 SD, a statistical outlier criterion (Barnett and Lewis 1984), then that mouse was considered AHA.

Instigation-heightened aggression

Neutral arena tests. A separate experimental procedure involved heightening aggression by the use of a social manipulation. Once aggressive behavior towards the intruder was reliably displayed at stable levels in the home cage, residents (n=9) had additional tests conducted in a neutral arena. The neutral arena was a 30×33×46 cm clear polycarbonate cage with clean pine shavings on the floor. The resident was removed from his home cage and placed into one corner of the neutral cage. Approximately 5 s later, an intruder was placed into the opposite corner. As with the home cage tests, neutral cage tests were terminated 5 min after the first attack bite. The pine shavings were changed after each encounter in the neutral cage. Tests in the neutral cage alternated with tests in the home cage, each test being separated from the next by 2–3 days. In comparison to the high levels of aggression engendered in the home cage, the levels of aggression in the neutral cage are ca. 50% lower (Miczek and O'Donnell 1978).

Instigation procedure. The instigation procedure began by placing an experimentally naive male intruder inside a protective shield – a clear, perforated, polycarbonate cylinder (18×6 cm). The shield containing the intruder, or "instigator", was placed into the center of the residents' home cage for a specific duration. The intruder and the protective shield were subsequently removed from the home cage and an aggression test in either the home or the neutral cage was performed immediately. In the first phase of the experiment, the duration of exposure was varied by 30 s, 2, and 5 min in order to assess its effects on aggression. Instigation test days were alternated with non-instigation control tests. Instigating intruders were replaced after two or three exposures to the resident.

Once an effective duration of instigation was determined, the persistence of instigation was examined by performing the aggression test either immediately (0 min), 15, or 60 min after the instigation procedure. Upon removal of the instigating intruder from the home cage, the resident was left alone for a specified time before a confrontation in the home cage. Each instigation test alternated with non-instigation control tests.

The specificity of the instigating stimulus was studied by using the following stimuli as potential "instigators" during a 5-min instigation period: (1) a breeding male; (2) a juvenile male; (3) an unfamiliar, pregnant female; (4) the empty cylinder. The stimuli were varied randomly across the residents and each test alternated with a home cage test.

Heightened aggression and CP-94,253

Separate groups of resident males were used to examine the effects of CP-94,253 on these two forms of heightened aggression. CP-94,253 (1.0–10.0 mg/kg) was administered IP, 15 min prior to either an oral injection of 1.0 g/kg ethanol (n=41) or 5 min of instigation by an intruder male (n=16). For the instigation procedure, aggressive behavior was tested in the neutral cage. Two tests were conducted each week, one following drug treatment and one following vehicle treatment. If a carryover effect from the previous drug test was apparent, as indicated by a change from baseline >20%, the vehicle test was repeated until the attack rates returned to baseline levels. The sequence of drug treatment varied randomly across individual residents.

Statistical analysis

All data were analyzed using a one-way Friedman repeated measures analysis of variance and, when appropriate, Student-Newman-Keuls multiple pairwise comparisons as post-hoc tests. The alpha level was set at 0.05. The ED_{50} was defined as the dose of CP-94,253 that produced a 50% reduction in behavior as compared to the baseline. The ED_{50} for individual mice was calculated by fitting the regression for the values of each dose. The group ED_{50} and 95% confidence intervals were determined based on the

mean and standard deviation of individual ED_{50} values. Non-overlapping confidence intervals were considered to be statistically significant.

Results

All male resident mice, housed as breeding pairs with a female, engaged in species-typical aggression. During the initial phase of each experiment, the mice exhibited stable and reliable baseline levels of fighting during semi-weekly confrontations with an intruder. These behaviors persisted to be displayed throughout the duration of the studies. As seen previously, the mice attacked with short latencies (i.e. 1–10 s), high frequencies (ca. 25), and low inter-trial variability (<20%).

Effects of CP-94,253 on species-typical aggression: antagonism by GR-127935

Species-typical aggression was dose-dependently reduced by administration of the 5-HT_{1B} agonist, CP-94,253, as measured by the frequency of attack bites (χ^2=36.38, P<0.001), sideways threats (χ^2=27.28, P<0.001) (Fig. 1) and tail rattles (χ^2=37.45, P<0.001), (data not shown). These anti-aggressive effects of CP-94,253 were significant at all doses (3–17 mg/kg) for attack bites and tail rattle frequencies. Sideways threats were significantly reduced by the 10.0 and 17.0 mg/kg doses of CP 94,253 (P<0.05).

When administered alone, GR 127935 (10 mg/kg) had no effect on the aggressive behaviors of these mice. Pretreatment with GR 127935 produced a right-ward shift in the dose-effect curve of the agonist's anti-aggressive effects. GR 127935 significantly increased the ED_{50} values by CP-94,253 for the reduction of attack bites from 7.2 mg/kg (95% CI=6.1, 8.4) to 14.5 mg/kg (95% CI=11.4, 17.5) and for sideways threats from 7.2 mg/kg (95% CI=6.1, 8.2) to 15.6 mg/kg (95% CI=13.1, 18.2); Fig. 1). Pretreatment with the 5-HT_{1A} antagonist, WAY 100,635 (0.1 mg/kg) did not alter the anti-aggressive effects of CP-94,253, (data not shown), nor was there an effect of this antagonist alone (Miczek et al. 1998a).

Behavioral specificity

The anti-aggressive effects of CP-94,253 were seen in the absence of any changes in either walking or rearing. In fact, a non-significant trend towards increased duration of walking and contact was observed at 3.0, 5.6 and 10.0 mg/kg CP-94,253 (Fig. 2). The duration of grooming, however, was decreased (χ^2=27.64, P<0.001) by all doses of CP-94,253. When administered alone, GR 127935 did not affect locomotor behavior. Following pretreatment with GR 127935, CP-94,253 (17.0, 30.0 mg/kg) decreased the duration of walking (χ^2=32.51, P<0.001), rearing (χ^2=28.84, P<0.001) and grooming

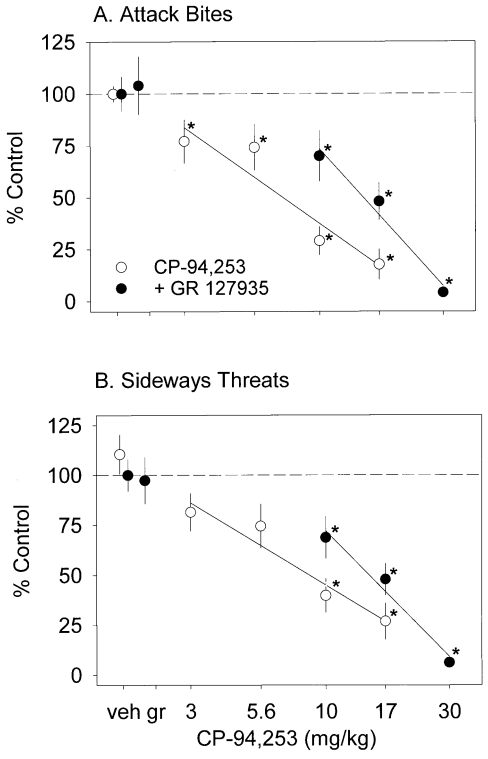

Fig. 1 **A** The effects of CP-94,253 on the frequency of attack bites. *Open symbols* represent data of the agonist alone. *Filled symbols* represent data of the agonist after pretreatment with GR 127935. Data are presented as percent change from baseline levels of means±1 SE (*vertical lines*). A regression line is shown, fitting the effects of CP-94,253 on this behavior. *Asterisks* denote significance as compared to vehicle (P<0.05). **B** The effects of CP-94,253 on the frequency of sideways threats. *Open symbols* represent data of the agonist alone. *Filled symbols* represent data of the agonist after pretreatment with GR 127935. Data are presented as percent change from baseline levels of means±1 SE (*vertical lines*). A regression line is shown, fitting the effects of CP-94,253 on this behavior. *Asterisks* denote significance as compared to vehicle (P<0.05)

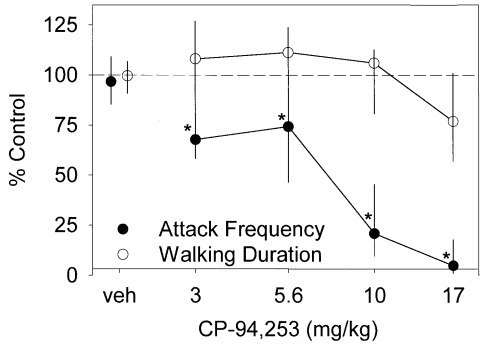

Fig. 2 The effects of CP-94,253 on the duration of walking (○) and on the frequency of attack bites (●), expressed as percent change from vehicle control levels. Medians and interquartile ranges (*vertical lines*) are shown. The *horizontal dotted line* denotes 100% of control. *Asterisks* indicate statistically significant effects as compared to vehicle (P<0.05)

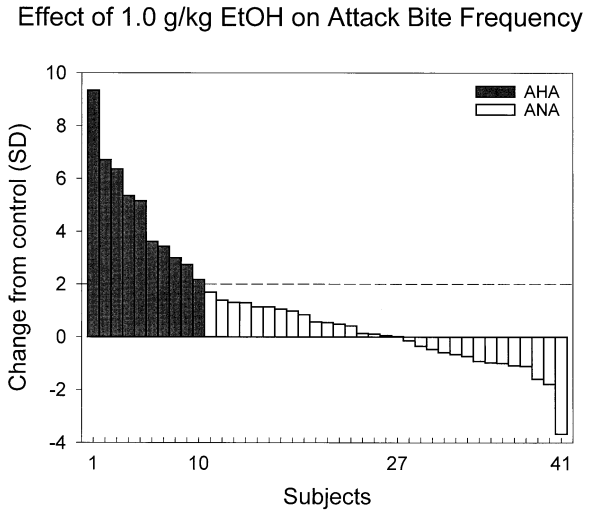

Fig. 3 Differences in the response to orally administered ethanol (1.0 g/kg) are indicated by the change in attack frequency of each individual mouse when compared to its vehicle control level. The *vertical bars* represent the change in attack frequency expressed as standard deviations. If the number of attacks exceeded the individual's vehicle control by 2 SD, then the mouse was considered to exhibit alcohol-heightened aggression (AHA *dark bars*); mice whose aggressive behavior did not meet this criterion were considered to be non-heightened (ANA *clear bars*)

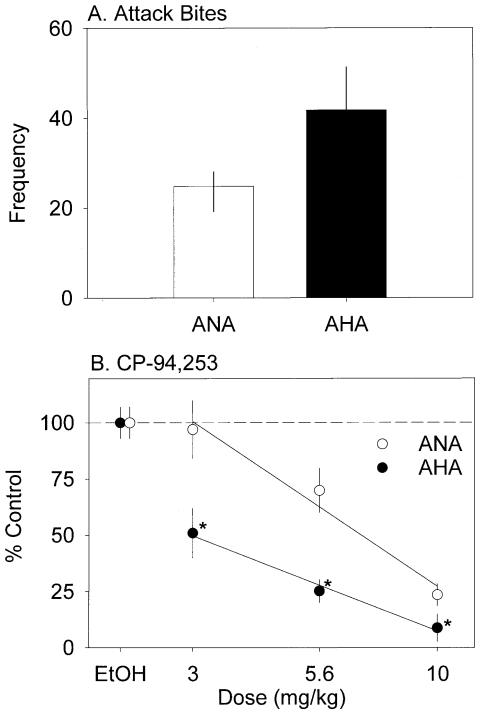

Fig. 4A, B The effects of CP-94,253 on alcohol-heightened (*filled symbols*) and alcohol non-heightened (*open symbols*) aggression. The aggressive behavior portrayed is the frequency of attack bites as percent of control of the individual vehicle baselines. Data are presented as means±SE (*vertical lines*). A regression line is shown, fitting the effects of CP-94,253 to this behavior. **A** Displays the effects of alcohol, expressed as medians and interquartile ranges, on the frequency of attack bites in AHA and ANA mice. *Asterisks* indicate significance compared to vehicle ($P<0.05$)

($\chi^2=20.52$, $P<0.001$). There were no significant effects of CP-94,253 on locomotor behaviors following pretreatment with WAY 100,635 (Miczek et al 1998a).

Heightened aggression after ethanol

A 1.0 g/kg dose of ethanol increased aggression by a minimum of 2 SD above the individual vehicle baselines in ten out of 41 mice (see Fig. 3). Those mice whose aggression increased by more than 2 SD from baseline levels were labeled as Alcohol-Heightened Aggression (AHA) animals. In contrast to its enhancement of aggression, this dose of ethanol produced no significant changes in any of the non-aggressive motor activities. The remaining mice, whose aggression was not altered by administration of ethanol, were labeled Alcohol-Non-heightened Aggression (ANA) mice. In these mice, alcohol did not affect any of the aggressive or non-aggressive behaviors.

Heightened aggression after instigation

Instigation significantly increased the aggressive behavior of all mice as indicated by greater frequencies of attack bites ($\chi^2=41.27$, $P<0.001$) and sideways threats ($\chi^2=41.30$, $P<0.001$), as compared to their baseline level in the neutral cage. The increase in aggressive behaviors was not accompanied by any significant changes in the duration of walking or rearing. However, instigation reduced the duration of grooming ($\chi^2=28.58$, $P<0.001$) as compared to the baseline levels ($P<0.05$).

Various social interactions were examined for their capacity to instigate aggression. Of the stimuli tested, group-housed, adult male intruders were effective at instigating aggression ($P<0.05$). The empty protective shield, the juvenile male mice, and the unfamiliar female mice did not significantly affect subsequent aggression. When a breeding male mouse was tested as the instigator, the amount of subsequent aggression was variable; three of the eight mice showed very high levels of instigated fighting, while the aggression of the other five mice was either suppressed or not altered.

Effects of CP-94,253 on alcohol-heightened aggression

Alcohol-heightened aggression was dose-dependently decreased by administration of CP-94,253 prior to ethanol: [attack bites ($\chi^2=29.29$, $P<0.001$); sideways threats ($\chi^2=29.29$, $P<0.001$); tail rattle ($\chi^2=26.45$, $P<0.001$)]. Post hoc tests revealed that the frequency of attack bites and sideways threats were significantly attenuated by all doses of CP-94,253 (3–10 mg/kg). The frequency of tail rattles decreased at the 10.0 mg/kg dose of CP-94,253. The ED_{50} for the anti-aggressive effects of CP-94,253 in the AHA mice was significantly lower than the ED_{50} in

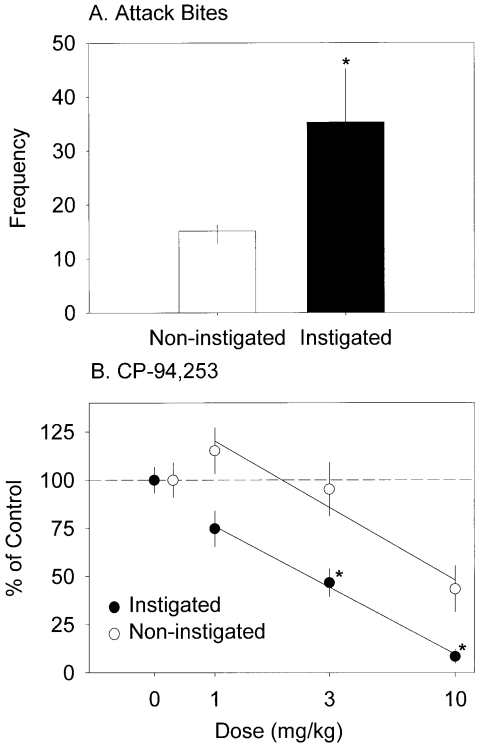

Fig. 5A, B The effects of CP-94,253 on instigated (*filled symbols*) and non-instigated (*open symbols*) aggression. The aggressive behavior portrayed is the frequency of attack bites as percent of control of the individual vehicle baselines. Data are presented as means±SE. A regression line is shown, fitting the effects of CP-94,253 on this behavior. **A** Illustrates the effects of instigation on the frequency of attack bites, expressed as medians and interquartile ranges. *Asterisks* indicate significance compared to vehicle ($P<0.05$)

the ANA mice, 3.8 mg/kg (95% CI=2.6, 5.1) versus 7.1 (95% CI=5.6, 8.7) mg/kg, respectively; see Fig. 4).

The locomotor behaviors of the AHA mice were dose-dependently reduced by CP-94,253: [walking (χ^2=15.04, $P<0.005$); rearing (χ^2=16.4, $P<0.003$); grooming (χ^2=15.84, $P<0.003$)]. A significant reduction in walking duration was observed at the 10.0 mg/kg dose ($P<0.05$) of CP-94,253, while 5.6 and 10.0 mg/kg decreased rearing duration ($P<0.05$). The duration of grooming by the AHA mice was not significantly affected by any dose of CP-94,253.

The effects of CP-94,253 in those mice whose aggressive behavior was not increased by ethanol (ANA) were closely similar to this drug's effects on species-typical aggression. Pretreatment with CP-94,253 caused significant decreases in the frequency of attack bites (χ^2=63.84, $P<0.001$), sideways threats (χ^2=61.58, $P<0.001$), tail rattles (χ^2=54.63, $P<0.001$) at the 5.6 and 10 mg/kg doses. Locomotor behaviors, [walking (χ^2=15.04, $P<0.005$), rearing (χ^2=19.55, $P<0.001$)] were reduced only by the 10.0 mg/kg dose ($P<0.05$); grooming duration (χ^2=14.8, $P<0.005$) was reduced by 5.6 and 10.0 mg/kg CP-94,253.

Effects of CP-94,253 on instigation-heightened aggression

Pretreatment with CP-94,253 attenuated instigation-heightened aggression as measured by the frequency of attack bites (χ^2=41.27, $P<0.001$) and sideways threats (χ^2=41.30, $P<0.001$) at the 3.0 and 10.0 mg/kg doses ($P<0.05$). The ED_{50} for the anti-aggressive effects of CP-94,253 on instigation-heightened aggression was significantly lower than the ED_{50} for baseline levels of aggression 2.7 mg/kg (95% CI=1.9, 3.5) versus 8.3 mg/kg (95% CI=6.3, 10.4); see Fig. 5). During instigated aggression, CP-94,253 reduced locomotor behavior: walking duration (χ^2=34.69, $P<0.001$); rearing duration (χ^2=13.36, $P<0.009$); and grooming duration (χ^2=28.58, $P<0.001$). Both the 3.0 and 10.0 mg/kg doses of CP-94,253 decreased the duration of walking ($P<0.05$), whereas rearing and grooming were decreased only at the 10.0 mg/kg dose ($P<0.05$).

The effects of CP-94,253 on non-instigated aggression were similar to the effect of this drug on other species-typical levels of aggression. CP-94,253 reduced the frequency of attack bites (χ^2=12.12, $P<0.007$), but only at the 10.0 mg/kg dose ($P<0.05$). Neither sideways threats nor tail rattles were not significantly affected by CP-94,253. Walking and rearing durations were not affected by CP-94,253. However, grooming duration (χ^2=9.46, $P<0.02$) was reduced by the 1.0 mg/kg dose of CP-94,253 ($P<0.05$).

Discussion

Agonists at the 5-HT_1 receptor family are confirmed to be highly effective anti-aggressive agents (Olivier et al. 1989b; Sanchez et al. 1993). Specifically, the present data strengthen the proposal that the 5-HT_{1B} receptor is particularly relevant to the modulation of aggression, as evidenced by the pharmacologically and behaviorally specific effects of CP-94,253 (Olivier et al. 1995). The anti-aggressive effects of this agonist are pharmacologically specific to the 5-HT_{1B} receptor as indicated by their antagonism by GR 127935, and this compound attenuates aggressive behavior in mice without a concurrent decrease in motor activity.

To date, CP-94,253 appears to be the most behaviorally specific 5-HT compound that reduces aggressive behavior. Though 5-HT_{1A} agonists such as 8-OH-DPAT and flesinoxan exert potent anti-aggressive effects (Olivier et al. 1989b; Mos et al. 1992; Sanchez and Hyttel 1994; de Almeida and Lucion 1997), the most effective doses are accompanied by locomotor suppression. Miczek et al. (1998b) reported that 0.3 mg/kg 8-OH-DPAT decreased the median walking duration to 31.5% of control, whereas the lower doses of CP-94,253 (1.0–5.6 mg/kg) tended to *increase* walking duration as compared to baseline levels. A slight decrease (80% of control, non-significant) was observed at the highest dose of CP-94,253.(17.0 mg/kg). Surprisingly, doses of

CP-94,253 that did not affect locomotor behaviors when administered alone, reduced these behaviors when administered following pretreatment with GR 127935. The potentiation of mild sedative effects by GR 127935 may support evidence for GR 127935 acting as a partial agonist to the $5HT_{1B/D}$ receptor (Pauwels 1997; Parsons et al. 1998).

Measurements of behavioral specificity should consider how the various acts and postures relate to each other and how a particular behavior requires a specific motoric demand (Krsiak 1975). Aggressive and non-aggressive behaviors are measured simultaneously within the same context and are part of a finite behavioral budget. As mice engage in high levels of aggressive sequences, they display fewer non-aggressive sequences such as rearing, walking, feeding, and grooming. Additionally, levels of aggressive behavior influence the degree of motor activation, and a drug that reduces aggression also potentially reduces the non-aggressive behaviors that occur during the aggression sequence. The salient non-aggressive behaviors measured in the current study differ in terms of specific muscle groups required to initiate and maintain each behavior highlighting the importance of incorporating multiple motor behaviors into a measure of behavioral specificity. One may propose that a desirable anti-aggressive agent is one that prevents exaggerated levels of aggression without disrupting species-typical behaviors. "As previously noted, concurrent assessment of locomotor and aggressive behavior is more informative than separate measurement of these behaviors (Miczek and Krsiak 1979)."

The specificity of the anti-aggressive properties of CP-94,253 in comparison to 8-OH-DPAT implies that the $5\text{-}HT_{1B}$ receptor may play a more specific role in the regulation of aggressive behavior than the $5\text{-}HT_{1A}$ receptor. Additional support for this hypothesis is drawn from earlier studies that report potent and relatively specific anti-aggressive effects of mixed $5\text{-}HT_{1A/B}$ agonists such as eltoprazine, TFMPP and mCPP (Olivier et al. 1990; Mos et al. 1992; Sanchez et al. 1993). Moreover, "knockout" mice lacking the gene encoding for the $5\text{-}HT_{1B}$ receptor exhibit shorter attack latencies and have higher frequencies of attack bites than either their wildtype or heterozygous counterparts (Saudou et al. 1994). However, the potent anti-aggressive properties of $5\text{-}HT_{1A}$ agonists suggest an interaction between 1A and 1B receptors as they modulate aggression.

Prior to the development of the selective $5\text{-}HT_{1B}$ agonist, CP-94,253, the mixed $5\text{-}HT_{1A/B}$ agonist, eltoprazine had been a most selectively acting anti-aggressive drug in the absence of sedation (Olivier et al. 1990). Presently, CP-94,253 stands out in terms of both pharmacological specificity to the $5\text{-}HT_{1B}$ receptor and behavioral specificity for aggressive behavior (Koe et al. 1992). In comparison with eltoprazine, CP-94,253 has a much greater affinity for the 1B receptor than for the 1A receptor in rat cerebral cortex (K_i=2.0±0.4 for inhibition of [^{3}H]5-HT binding and 4.4±0.9 nM for inhibition of [I]ICP binding versus 89±15 nM for the inhibition of [^{3}H]OH-DPAT binding). Although the $5\text{-}HT_{1B}$ receptors are not identical in rodents and in humans, the rodent 1B receptors are thought to be functionally homologous with the $h5\text{-}HT_{1B}$ receptors (Adham et al. 1992; Price et al. 1996; Schlicker et al. 1997). Both receptor types have similar distribution across species (Hoyer et al. 1994) and differ only in a single amino acid (Oksenberg et al. 1992; Martin and Humphrey 1994), making pre-clinical investigations of the $5\text{-}HT_{1B}$ receptor relevant for therapeutic interventions directed towards the $h5\text{-}HT_{1B}$ family.

The current methodologies attempted to engender high, transient levels of aggression in order to relate these models more closely to clinical concerns. One of these methods is the heightened aggressive behavior that is observed in several species following treatment with low to moderate doses of ethanol (Raynes and Ryback 1970; Chance et al. 1973; Miczek and Barry 1977; Peeke and Figler 1981; Blanchard et al. 1987; Miczek et al. 1992). In mice, a subgroup of individuals (ca. 20%), labeled AHA, show robust and reliable enhancement of aggressive behaviors when given a 1.0 g/kg dose of ethanol (Miczek et al. 1993, 1998a, 1998b). The 2 SD criterion that is used to identify this population is based on the principle that AHA animals represent statistical outliers (Barnett and Lewis 1984). In rats, alcohol heightened aggression can be brought about by either experimenter-administered or self-administered oral ethanol (van Erp and Miczek 1997). In view of the relevance to the human condition, it would be desirable to study alcohol heightened aggression in animals that self-administer alcohol.

Very high levels of attack behavior may be generated using the current instigation procedure, and this intense form of aggression has been attributed to high "aggressive arousal" (Berkowitz 1993). Social provocations can increase aggression as demonstrated prominently by attack "priming" and prolonged sensory contact (Potegal 1984; Potegal and Tenbrink 1984; Kudryavtseva 1991). Instigation seems to result from increased arousal that is specific to aggression rather than generalized as is indicated by no changes in locomotor behavior. Furthermore, the social provocations that increase inter-male aggression seem to be specific to those stimuli that typically evoke inter-male aggression and not other behaviors such as copulation and feeding (Thor and Carr 1979; Thor and Flannelly 1979; Potegal 1984, 1991). In the current study, only adult male mice (group-housed or breeding male mice) instigated aggression; stimuli not associated with inter-male aggression (i.e. the empty shield, the juvenile male, and the female mice) were ineffective to provoke higher levels of aggression. An important contrast between alcohol-heightened aggression and instigation relates to individual differences. Instigation proved effective in all mice, whereas alcohol's aggression enhancing effects are restricted to a subset of mice. The possibility that the neurochemical characteristics of mice instigated to high levels of "aggressive arousal" are similar to those of mice identified as AHA is an important topic of our ongoing work.

The current use of these two procedures enables comparison of 5-HT modulation of species-typical and extreme forms of aggressive behavior. Interestingly, the ED_{50}s for the anti-aggressive effects of CP-94,253 in alcohol-heightened and instigation-heightened aggression were similar (3.8 and 2.7 mg/kg, respectively), ca. 2-fold different from those doses that were effective on species-typical aggression (7.1 and 8.3 mg/kg, respectively). This comparison suggests that the higher rates of aggressive behavior are more sensitive to the effects of CP-94,253.

The relationship between alcohol and serotonin is interesting because of the interaction of these two substances at both the neurochemical and behavioral levels (McBride et al. 1993). It has been found that individuals who have a genetic predisposition to drink alcohol tend to have lower than average levels of CSF 5-HIAA and exhibit tendencies towards impulsive violent behavior (Virkkunen and Linnoila 1993; Higley et al. 1996). These correlations suggest that there may be a genetic component for aggressive behavior that is elevated by alcohol and that both of these variables, aggressive behavior and alcohol intake, may be influenced by serotonin. This hypothesis is further supported by linkage between antisocial alcoholics and polymorphisms of the $5HT_{1B}$ gene (Lappalainen et al. 1999). We are currently investigating the neuropharmacological characteristics of AHA mice under resting and challenge conditions.

In conclusion, CP-94,253 produced a behaviorally specific reduction in both species-typical and two forms of heightened aggressive behavior. Heightened aggression was reduced by lower doses of CP-94,253 than were required to reduce species-typical aggression, suggesting that the $5HT_{1B}$ receptors may be preferentially involved in the control of high levels of aggressive behavior. Moreover, the highly specific behavioral effects of CP-94,253 make this type of compound particularly intriguing as a potential pharmacotherapeutic option. Using the currently introduced methodology, it may now be possible to investigate how the $5\text{-}HT_{1B}$ receptor modulation of high levels of aggression relates to its role in other pathologies such as impulse discontrol and drug abuse (Lucas et al. 1997; Maurel et al. 1998; Parsons et al. 1998; Rocha et al. 1998).

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