Psychopharmacology (2004) 172:255–263
DOI 10.1007/s00213-003-1661-1

ORIGINAL INVESTIGATION

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$GABA_A/\alpha_1$ receptor agonists and antagonists: effects on species-typical and heightened aggressive behavior after alcohol self-administration in mice

Received: 12 February 2003 / Accepted: 18 September 2003 / Published online: 25 November 2003

Abstract *Rationale:* The positive modulation of gamma-aminobutyric acid type-A ($GABA_A$) receptors is a putative mechanism via which alcohol escalates aggressive behavior. Broad-spectrum benzodiazepine antagonists block alcohol-heightened aggression in rats and monkeys. However, the degree to which $GABA_A$ subunit composition plays a role in heightened aggressive behavior induced by self-administration of a moderate alcohol dose remains unresolved. *Objective:* β-Carboline-3-carboxylate-t-butyl ester (β-CCt) and zolpidem act preferentially at $GABA_A$ receptors containing the α_1 subunit as antagonist and agonist, respectively, and serve as useful tools to evaluate the role of $GABA_A$ receptor subtypes in self-administered alcohol on aggression. *Methods:* Male resident mice, housed in breeding pairs, were conditioned to nose-poke in a removable panel in their home cage, with each fifth poke being reinforced by the delivery of 0.05 ml of 6% ethanol (EtOH). After consuming EtOH, the resident mice were given the antagonists β-CCt and flumazenil or agonists zolpidem and triazolam, and then confronted an intruder male in their home cage for a 5-min period. *Results:* Following self-administration of EtOH (1.0 g/kg, 1.7 g/kg), 14 of 37 resident mice displayed unusually large increases in the frequency of attack bites and sideways threats. Flumazenil or β-CCt decreased alcohol-heightened and non-heightened aggression in a dose-dependent manner. Administration of 3 mg/kg β-CCt lowered the aggression-heightening effects of 1 g/kg and 1.7 g/kg EtOH, but did not antagonize the sedative effects of 3.0 g/kg EtOH. Triazolam and zolpidem decreased alcohol-heightened and non-heightened aggressive behavior, and these anti-aggressive effects were accompanied by reduced motor activity, indicating sedation. *Conclusions:* Benzodiazepine antagonists, particularly those acting preferentially at $GABA_A/\alpha_1$ subunit-containing receptors, decrease alcohol-heightened and species-typical aggressive behavior, but are ineffective in attenuating the sedative effects of alcohol.

Keywords GABA receptors · Aggression · Alpha subunits · Self-administration · Benzodiazepine receptor · Alcohol · Hypnotic · Locomotion

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Introduction

Among the neurobiological mechanisms through which alcohol achieves its behavioral and physiological effects, ionophoric receptors have emerged as important candidate sites (Grobin et al. 1998; Harris et al. 1998). Gamma-aminobutyric acid type-A ($GABA_A$) receptors appear particularly relevant to the aggression-heightening effects of alcohol in experimental models with several animal species (Miczek et al. 1993, 2002; Grobin et al. 1998). Alcohol acts as a positive modulator at $GABA_A$ receptors, increasing GABA-induced inhibitory currents and increasing chloride flux by longer and more frequent Cl^- channel openings (Nestoros 1980; Suzdak et al. 1986; Mehta and Ticku 1988). Early preclinical evidence showed that positive modulators of the $GABA_A$ receptors, such as benzodiazepines, barbiturates, and neurosteroids,

share not only alcohol's anxiolytic, sedative, hypnotic, and amnesic effects, but also its aggression-heightening effects (Miczek and Krsiak 1979).

Characteristically, alcohol as well as other positive modulators of the $GABA_A$ receptor increase the frequency and duration of aggressive behavior far in excess of the species-typical level at lower and moderate doses, whereas sedative effects prevail at higher doses (Raynes and Ryback 1970; Chance et al. 1973; Miczek and Barry 1977; Peeke et al. 1981; Blanchard et al. 1987; Weerts and Miczek 1996; Van Erp and Miczek 1997). While this alcohol effect is large in magnitude, it is consistently restricted to approximately 20–30% of all animals tested (Miczek et al. 1992, 1998). Moreover, concurrent administration of $GABA_A$-positive receptor modulators with alcohol results in additive enhancement of aggressive behavior. For example, largely additive effects occur when combining low doses of alcohol with allopregnanolone (Fish et al. 2001) and chlordiazepoxide (Miczek and O'Donnell 1980). Conversely, benzodiazepine receptor antagonists such as flumazenil effectively attenuate the aggression-heightening effects of alcohol (Weerts et al. 1993).

$GABA_A$ receptors are heteropentameric proteins consisting of subunits derived from at least seven different families, many with multiple variants (Rudolph et al. 2001). The exact subunit composition of a $GABA_A$ receptor can determine its sensitivity to such positive modulators as diazepam or allopregnanolone (Lambert et al. 2001), and it may be postulated that specific subunit composition also is of significance for different actions of alcohol. With regard to the effects of benzodiazepine-type drugs, recent studies with gene-targeted point-mutated mice have pointed to the $GABA_A/\alpha_1$ receptor as critical for the sedative effects of diazepam (Rudolph et al. 1999; McKernan et al. 2000), whereas the $GABA_A/\alpha_2$ receptor has been linked to anxiolytic-like effects of this benzodiazepine (Löw et al. 2000). Compounds that act preferentially at specific $GABA_A$ receptor subtypes have been shown to attenuate the reinforcing effects of alcohol when microinjected into discrete brain structures such as the ventral pallidum ($GABA_A/\alpha_1$) or hippocampal ($GABA_A/\alpha_5$) sites of alcohol-preferring rats (June et al. 2001; Harvey et al. 2002). β-Carboline-3-carboxylate-t-butyl ester (β-CCt) is an antagonist with 20-fold selectivity for $GABA_A/\alpha_1$ receptors (Huang et al. 2000), and this compound offers the opportunity to evaluate whether or not alcohol-heightened aggressive behavior can be antagonized by the $GABA_A/\alpha_1$ receptor subtype. Zolpidem is an imidazopyridine compound that is characterized by a sixfold or greater selectivity for $GABA_A/\alpha_1$ receptors over other $GABA_A$ receptor subtypes (Sanger and Zivkovic 1986; Damgen and Lüddens 1999; Rudolph et al. 2001). In preliminary experiments, zolpidem was found to effectively sedate aggressive animals. This compound did not increase aggressive behavior at low doses as is characteristic for most nonselective benzodiazepines such as midazolam, diazepam and chlordiazepoxide (Miczek 1974; Miczek and O'Donnell 1980; Rodgers and Waters 1985; Gourley et al. 2002).

The purpose of the present experiments was to systematically investigate the role of the $GABA_A/\alpha_1$ receptor in mediating the increase in aggression induced by self-administered alcohol. We evaluated the extent to which β-CCt (the $GABA_A/\alpha_1$ receptor-preferring antagonist) relative to flumazenil (a broad-spectrum antagonist) reduced alcohol-heightened aggressive behavior and determined the specificity of this anti-aggressive effect. In addition, we assessed the effects of the $GABA_A/\alpha_1$ receptor-preferring agonist zolpidem as well as the nonspecific benzodiazepine triazolam on alcohol-heightened aggressive behavior.

Methods and materials

Subjects

Pairs of adult male and female CFW mice (Charles River Laboratories, Wilmington, MA), weighing between 22 g and 25 g on arrival, were housed in clear polycarbonate cages (28×17×14 cm). Pups were weaned at 3 weeks of age. The males of the breeding pairs served as resident animals (*vide infra*). Additional male CFW mice were housed in groups of 8–10 in large cages (46×24×15 cm). The group-housed male mice served as stimulus intruder animals, as specified below. The floor of each cage was covered with wood-chip bedding. Purina rodent chow and water were freely available through stainless-steel wire lids. Upon arrival from the breeder, all animals were allowed to acclimate to the laboratory environment for 7 days. The mice were housed in a room with controlled temperature at 22±1°C; humidity 30–40% and on a 12-h/12-h light/dark cycle. The Tufts University Animal Care and Use Committee supervised and approved all housing and experimental procedures, following the Guide for the Care and Use of Laboratory Animals (National Research Council 1996).

Apparatus and measurements

All experimental testing for alcohol self-administration and for aggressive behavior occurred in the home cage of the resident mice, except when specified otherwise.

Ethanol self-administration

The experimental set-up and conditioning procedure was described and illustrated previously (Miczek and de Almeida 2001). Briefly, an experimental panel was inserted into the middle of the mouse cage and affixed to the side walls with two thumb screws at the start of each session. On the left and right sides of the panel, operanda in the form of nose poke sensors were mounted 3 cm above the floor with stimulus lights 5 cm above. In the center of the panel, a cup for fluid delivery was located in a recess and connected to a syringe pump. A house light provided illumination (all devices were from Med Associates, St. Albans, Vt.). The devices in the panel as well as the pump were controlled by a PC interface and associated Windows Med-PC software.

Initially, access to drinking fluid was restricted overnight, and each nose poke by the resident mouse was reinforced with the delivery of 0.05 ml sucrose (10%) (fixed ratio schedule of reinforcement; FR 1). Using a sucrose fading procedure, similar to one developed for rats (Grant and Samson 1985; Samson 1986), the FR requirement was increased gradually to 5 in the course of daily 30-min sessions. Ethanol was added to the sucrose solution in1% steps up to 6%. Thereafter, the sucrose solution was gradually

decreased from 10% to 0%. Each mouse was adapted to sucrose in the drinking fluid, and subsequently acquired the nose-poke response within minutes of the first experimental session. After the ethanol was faded in and the sucrose was faded out, the daily experimental sessions were reduced to a length that allowed the animals to consume 1 g/kg ethanol, usually requiring less than 3 min, with a maximal limit of 30 min.

Aggressive behavior in resident–intruder confrontation

Aggressive behavior was engendered in the resident male mice during confrontations with an intruder male (Miczek and O'Donnell 1978). Initially, the baseline level of aggressive behavior was allowed to stabilize during six confrontations that were conducted 6 h after the ethanol self-administration session, each confrontation 3–4 days apart. Once the resident's rate of attacks toward the intruder varied less than 15%, the confrontations were transferred to a large neutral cage (38×33×16 cm). Under these conditions (de Almeida and Miczek 2002), the resident attacked the intruder ca. 15–20 times in a 5-min confrontation.

For the remainder of the experiment, twice-weekly sessions consisted of ethanol self-administration followed 15 min later by a confrontation of the resident male mouse with an intruder in a neutral cage. The behavior of the mice was recorded on videotape, using a 0.5-lux camera and a video cassette recorder. At a later time, the videotapes were analyzed, and the frequency and duration of salient elements of aggressive and non-aggressive behaviors were measured by a trained observer, using the custom-designed data acquisition system similar to that described previously (Miczek 1982). The salient elements of aggressive behaviors are defined and illustrated previously (Miczek and O'Donnell 1978) and comprised the following: pursuit, sideways threat, bite, and tail rattle. Non-aggressive behaviors were recorded as well, and comprised the following: anogenital contact with the intruder, grooming, walking, and rearing. Inter- and intra-observer reliability for encoding the frequency and duration of each of these behaviors were calculated using the Spearman correlation coefficient and ranged from 0.95 for the duration of walking to 0.98 for the frequency of attack bites.

Drugs

Ethanol (AAPER Alcohol, Shelbyville, KY) was prepared as a 1–6% w/v solution. Flumazenil, β-CCt, zolpidem, triazolam were dissolved in Tween 80 (1%), propylene glycol (14%), and distilled water (85%). Flumazenil was a gift from Hoffman-LaRoche Pharmaceuticals (Nutley, NJ), zolpidem was a gift from Sanofi-Synthelabo (Bagneux, France), β-CCt was synthesized at the University of Wisconsin-Milwaukee, as described in detail previously (Cox et al. 1995). Triazolam was purchased from Sigma-RBI (St. Louis, MO). These latter four drugs were injected intraperitoneally (i.p.) in a volume of 1 ml/100 g body weight.

Experimental design

Determination of alcohol-heightened aggression

The first experiment was designed to determine those individual mice that consistently exhibit alcohol-heightened aggressive behavior. Immediately before each of six consecutive confrontations, two per week, resident mice (n=37) self-administered either 1 g/kg ethanol or water in alternate sessions. If the rate of attacks after ethanol self-administration exceeded that after water self-administration by at least two standard deviations, i.e., fulfilled the statistical outlier criterion, the individual was categorized as alcohol-heightened aggressor (AHA). The remaining mice were designated as alcohol non-heightened aggressors (ANA).

Determination of ethanol dose–effect function

In the course of the next 2 weeks, the dose of self-administered ethanol was varied systematically prior to the confrontations with the intruder male. In two sessions per week, each separated by 3–4 days, the resident male self-administered either 0.6, 1.0, 1.7 or 3.0 g/kg ethanol. Ethanol doses were varied by adjusting the number of deliveries of 6% ethanol according to the body weight of the individual mouse. Only 10 of 37 mice completed enough responses to achieve the 3.0-g/kg dose in at least one test.

Treatment with β-CCt or flumazenil

Two series of experiments were designed in order to assess the effects of β-CCt on aggressive behavior in AHA (n=8) and ANA mice (n=11). In the first experiment, the resident male mice (n=19) self-administered 1 g/kg ethanol during daily sessions and once a week, they were treated with a dose of β-CCt (0.3–10 mg/kg, i.p.) or flumazenil (3–10 mg/kg, i.p.) immediately after completing the ethanol self-administration. During a second test per week, the ethanol self-administration session was followed by an injection with vehicle. Fifteen minutes after drug or vehicle injection, the resident male mouse was placed into the neutral test cage with an intruder for a 5-min confrontation.

During a second experiment, the resident male mice (n=19) self-administered 1.0 g/kg ethanol during daily sessions. Once a week, the dose of ethanol was varied from 0.6 g/kg to 3.0 g/kg and at the completion of this session, 1.0 mg/kg β-CCt was administered. As before, during the alternate test of the week, the vehicle was administered after the alcohol self-administration session. Fifteen minutes later, the aggression test was conducted in the neutral cage in both AHA (n=8) and ANA mice (n=11).

Treatment with zolpidem or triazolam

Two series of experiments were designed to assess the effects of zolpidem and triazolam on aggressive behavior in AHA and ANA mice. Zolpidem (0.1, 0.3 and 1.0 mg/kg), triazolam (1, 3, 10 and 30 μg/kg) or the vehicle were administered immediately after the resident mouse had self-administered 1 g/kg ethanol, and 15 min later the confrontation with the intruder was scheduled in both AHA (n=6) and ANA (n=12) mice. As before, drug and vehicle injections alternated before the two weekly resident-intruder tests.

Data analysis

All data for dose–effect and drug interaction studies were analyzed using one-way repeated-measures analyses of variance (ANOVA) except for the second βCCt–EtOH interaction experiment, where two-way ANOVAs were performed. When appropriate, Bonferroni post-hoc t-tests were used to compare individual drug doses with average vehicle control values. The effects of the 3-g/kg ethanol dose were analyzed using a paired t-test, since only a subset of mice completed self-administering this dose within 30 min. The alpha level was set at 0.05. The ED_{50} was defined as the dose of triazolam, zolpidem, β-CCt and flumazenil that produced a 50% reduction in behavior relative to the baseline. The ED_{50} was calculated by linear regression analysis based on the mean values of each dose.

Results

Alcohol-heightened aggressive behavior

On average, resident mice self-administered 1 g/kg ethanol in 2.6 min. Of 37 resident mice, 14 exceeded

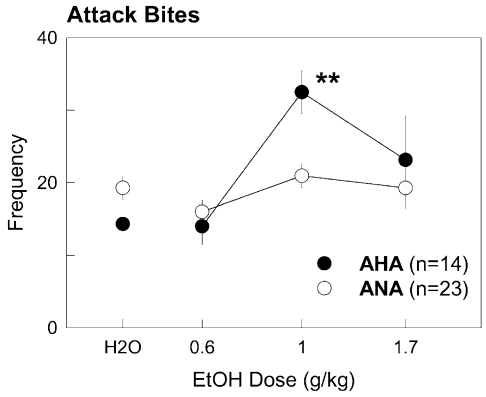

Fig. 1 Frequency of attack bites as a function of self-administered ethanol dose (g/kg) in male resident mice confronting an intruder. The mice were categorized as alcohol-heightened aggressors (AHA, n=14) or as alcohol non-heightened aggressors (ANA, n=23) on the basis of alternating tests involving self-administration of the water vehicle or 1 g/kg ethanol. Statistically significant changes from the water vehicle control data are identified by *asterisks* (** P<0.01)

the statistical outlier criterion in rate of attack behavior after self-administering 1 g/kg ethanol, i.e., engaged in alcohol-heightened aggression. When the dose of ethanol was varied in the self-administration session before the confrontation with the intruder, increases in the frequency of attack bites and pursuits followed the consumption of ethanol in AHA mice (attack bites: $F_{3,13}$=8.66, P<0.001; t=4.28, P<0.001 for 1.0 g/kg; pursuit: $F_{3,13}$=3.60, P=0.02; t=3.24, P=0.008 for 1.7 g/kg; Fig. 1). No significant changes in non-aggressive behavior were seen at the 1.0-g/kg and 1.7 g/kg ethanol doses. Only 5 AHA and 5 ANA animals completed the requirements to self-administer 3.0 g/kg within 30 min, and most of those who did exhibited low rates of aggressive behavior.

Effects of β-CCt and flumazenil on aggressive behavior in AHA and ANA mice

In the first experiment, administration of β-CCt after self-administration of 1 g/kg ethanol reduced the frequency of attack bites, sideways threats, pursuits, both in AHA and ANA mice (AHA: $F_{4,7}$=3.34, 4.99, and 7.19, P<0.02 and lower; ANA: $F_{4,10}$=7.79 and 9.01, P<0.001). Specifically, these anti-aggressive effects were isolated to the 3.0 mg/kg and 10.0 mg/kg β-CCt doses (attack bites, sideways threats; Fig. 2). The non-aggressive behaviors (walking, rearing, grooming) were not systematically affected by β-CCt, neither in AHA nor in ANA mice, except for a decrease in the frequency of walking at the 10 mg/kg β-CCt dose (P<0.001; data not shown).

Administration of flumazenil after self-administration of 1 g/kg ethanol decreased the frequency of attack bites and sideways threats, both in AHA and ANA mice (AHA: $F_{3,7}$=5.04 and 4.021; ANA: $F_{3,10}$=6.67 and 8.38, P<0.02 and higher). Specifically, the reduction in these aggressive behaviors was significant after administration of the 10 mg/kg flumazenil dose in AHA and ANA mice (AHAs: bites t=3.41, P=0.008, threats t=3.71, P=0.004; ANAs: bites t=3.79, P=0.002, threats t=3.16, P=0.011), and also after the 5.6 mg/kg dose for sideways threats in AHA mice (t=2.74, P=0.037; Fig. 3). Flumazenil left the non-aggressive activities (grooming, rearing, walking) largely unaffected in both types of mice after alcohol self-administration, except for a modest, but statistically significant decrease in walking in ANA mice at the 10 mg/kg dose (walking frequency t=2.61, P=0.042; duration t=2.88, P=0.022).

In a second experiment, when 1 mg/kg or 3 mg/kg β-CCt was administered after the self-administration of various doses of ethanol (0.6, 1.0, 1.7 g/kg), the aggression-heightening effects of ethanol were blocked (EtOH × β-CCt interaction $F_{4,25}$=3.41, P=0.023; Fig. 4).

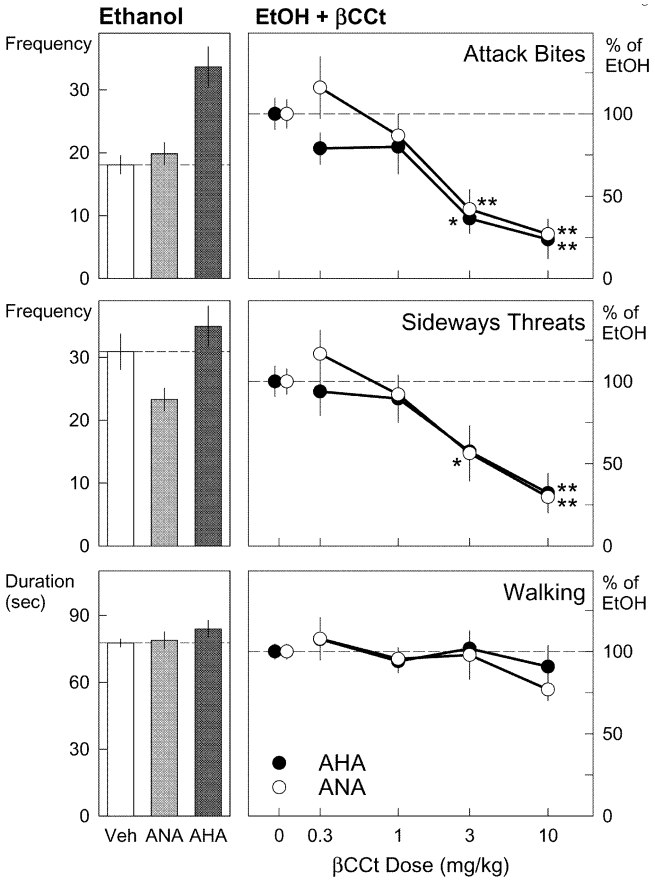

Fig. 2 *Left* Frequency of attack bites (*top*), sideways threats (*middle*) and duration of walking (*bottom*) after self-administering the water vehicle (*clear bar*) or 1 g/kg ethanol in ANA (n=11, *grey bar*) or AHA mice (n=8, *dark bar*). *Right* Percentage change in attack bites, sideways threats and duration of walking as a function of administration of β-CCt doses (mg/kg) in AHA (*filled circle*) and ANA (*clear circle*) mice after they had self-administered 1.0 g/kg ethanol. The control levels for the ethanol–β-CCt interaction studies were the values obtained during tests with 1.0 g/kg ethanol self-administration plus β-CCt vehicle. Statistically significant changes from the water vehicle control data are identified by *asterisks* (*P<0.05; **P<0.01)

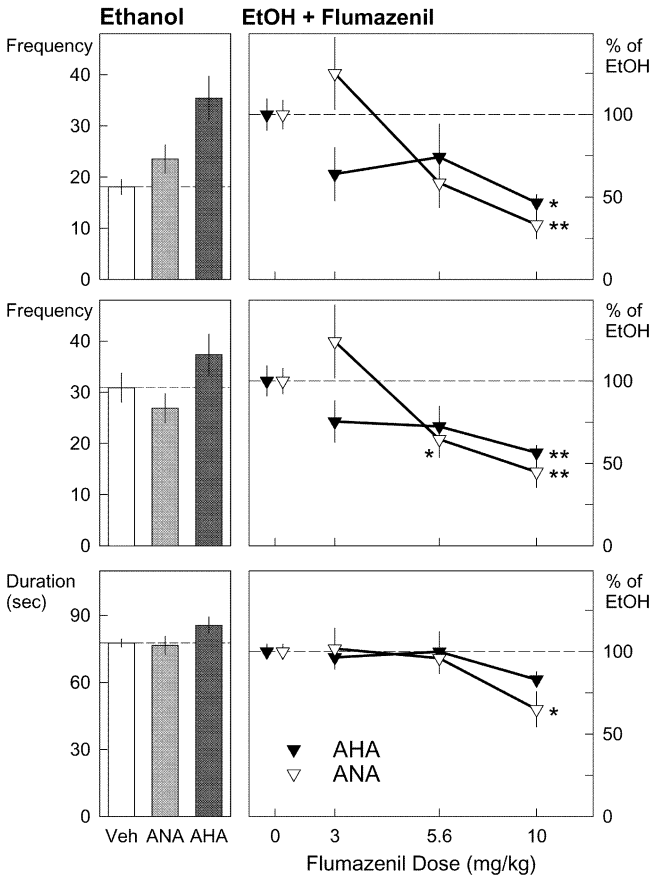

Fig. 3 *Left* Frequency of attack bites (*top*), sideways threats (*middle*) and duration of walking (*bottom*) after self-administering the water vehicle (*clear bar*) or 1 g/kg ethanol in ANA (n=11, *grey bar*) or AHA mice (n=8, *dark bar*). *Right* Percentage change in attack bites, sideways threats and duration of walking as a function of administration of flumazenil doses (mg/kg) in AHA (*filled triangles*) and ANA (*clear triangles*) mice after they had self-administered 1.0 g/kg ethanol. The control levels for the ethanol–flumazenil interaction studies were the values obtained during tests with 1.0 g/kg ethanol self-administration plus flumazenil vehicle. Statistically significant changes from the water vehicle control data are identified by *asterisks* (*P<0.05; **P<0.01)

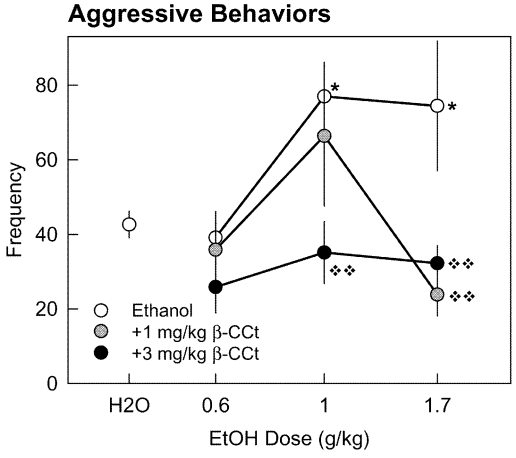

Fig. 4 Frequency of aggressive behaviors comprising attack bites, sideways threats and pursuits as a function of self-administered ethanol dose in male resident mice confronting an intruder. The measurements were obtained from the AHA mice (n=8) after they had self-administered various doses of ethanol only and then confronted an intruder (*clear circle)* or after ethanol self-administration and treatment with 1 mg/kg β-CCt (i.p.; *grey circle*) or 3 mg/kg β-CCt (*black circle*). For comparison, the level of attack bites and sideways threats after water self-administration, as determined in the initial experiment, is shown. The *asterisks* denote significant differences between the values from tests after alcohol self-administration and after water vehicle consumption (P<0.05), and *diamonds* indicate significant differences (P<0.01) between the values from alcohol effects in the presence and absence of β-CCt

Specifically, the frequency of the salient elements of aggressive behavior (pursuits, attack bites and sideways threats), shown as a summary measure was significantly increased after self-administering 1.0 g/kg or 1.7 g/kg ethanol. Pretreatment with 1 mg/kg β-CCt reduced the increase after 1.7 g/kg ethanol (t=3.89, P=0.001), and pretreatment with 3 mg/kg β-CCt reduced the increase in aggressive behavior after both 1.0 g/kg (t=3.55, P=0.003) and 1.7 g/kg ethanol (t=3.51, P=0.003). Only five resident mice self-administered the 3.0 g/kg ethanol dose within the 30-min session, and treatment with 3.0 mg/kg β-CCt did not reverse the aggression-suppressing and motor-slowing effects of the high ethanol dose in the mice that finished the session and those that did not (data not shown). Administration of 3.0 mg/kg β-CCt did not reverse the suppressive effects of higher ethanol doses on the frequency of attack bites, sideways threats, pursuits and tail rattles in ANA mice. When administered with β-CCt, neither ANA nor AHA mice showed significant changes in non-aggressive behavior such as anogenital contact, grooming, walking, rearing (data not shown).

Effects of β-CCt and flumazenil on ethanol-reinforced responding

Administration of β-CCt before the 15-min session of ethanol-reinforced responding tended to reduce the rate of responses by 13.6% and 33.6% (3 mg/kg and 10 mg/kg, respectively, P<0.12), and the effects of flumazenil (1–10 mg/kg) on ethanol-reinforced responding were not significant (data not shown).

Effects of zolpidem and triazolam on aggressive behavior in AHA and ANA mice

After self-administration of 1 g/kg ethanol, administration of zolpidem decreased aggressive and non-aggressive behavior significantly both in AHA and ANA mice ($F_{3,11}$=5.31, P=0.01 and 16.62, P<0.001 for attacks and $F_{3,11}$=5.61, P=0.001 and 16.18, P<0.001 for sideways threats), and this effect was entirely due to the suppressive effects of the high 1 mg/kg dose (Fig. 4). Specifically, the frequencies of sideways threat and attack bites in AHA mice were significantly lower after treatment with 1 mg/

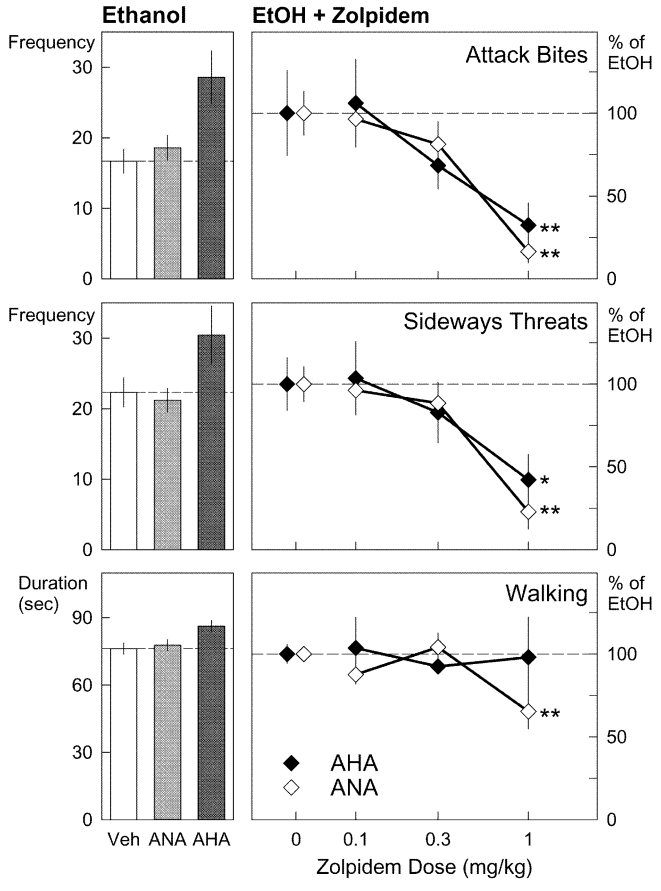

Fig. 5 *Left* Frequency of attack bites (*top*), sideways threats (*middle*) and duration of walking (*bottom*) after self-administering the water vehicle (*clear bar*) or 1 g/kg ethanol in ANA (n=12, *grey bar*) or AHA mice (n=8, *dark bar*). *Right* Percentage change in attack bites, sideways threats and duration of walking as a function of administration of zolpidem doses (mg/kg) in AHA (*filled diamonds*) and ANA (*clear diamonds*) mice after they had self-administered 1.0 g/kg ethanol. The control levels for the ethanol–zolpidem interaction studies were the values obtained during tests with 1.0 g/kg ethanol self-administration plus zolpidem vehicle. Statistically significant changes from the water vehicle control data are identified by *asterisks* (*P<0.05; **P<0.01)

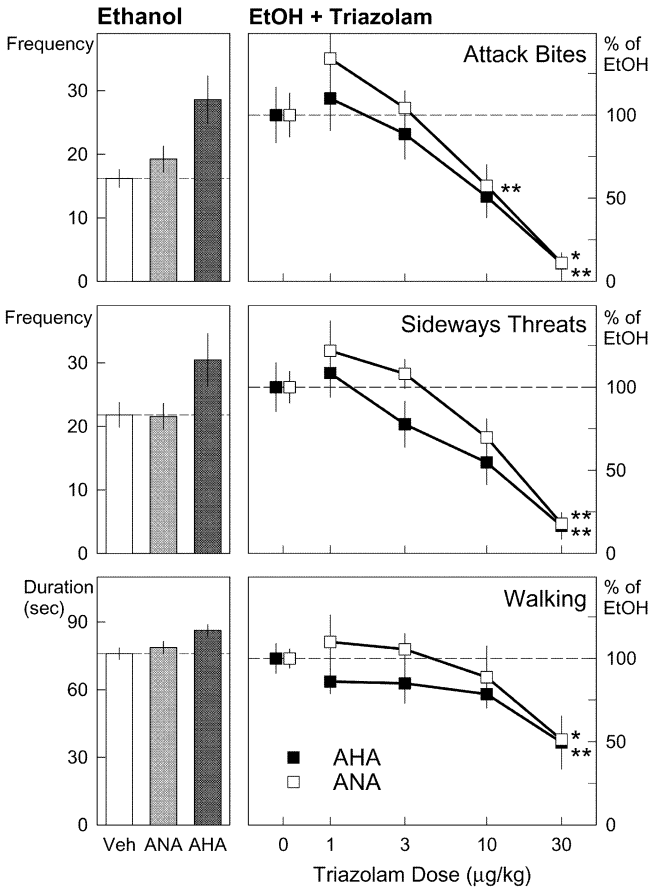

Fig. 6 *Left* Frequency of attack bites (*top*), sideways threats (*middle*) and duration of walking (*bottom*) after self-administering the water vehicle (*clear bar*) or1 g/kg ethanol in ANA (n=12, *grey bar*) or AHA mice (n=6, *dark bar*). *Right* Percentage change in attack bites, sideways threats and duration of walking as a function of administration of triazolam doses (mg/kg) in AHA (*filled squares*) and ANA (*clear squares*) mice after they had self-administered 1.0 g/kg ethanol. The control levels for the ethanol–triazolam interaction studies were the values obtained during tests with 1.0 g/kg ethanol self-administration plus triazolam vehicle. Statistically significant changes from the water vehicle control data are identified by *asterisks* (*P<0.05; **P<0.01)

kg zolpidem, as were the frequencies of sideways threat, attack bites, and tail rattles (Fig. 5). Administration of 1 mg/kg zolpidem significantly reduced the frequency and duration of rearing in AHA and ANA mice by approximately 50% ($F_{3,11}$=4.22, P=0.02), and decreased the frequency and duration of walking in ANA mice only ($F_{3,11}$=14.97, P=0.01).

Similarly, administration of triazolam decreased aggressive and non-aggressive behavior in AHA and ANA mice ($F_{3,11}$=9.12 and 18.79, P<0.001 for attacks, Fig. 6). Specifically, 30 μg/kg triazolam significantly reduced the frequency of sideways threats (t=4.90 and t=6.11, P<0.001) and attack bites (t=5.03 and t=6.03, P<0.001), and in ANA mice the 10 μg/kg dose was also effective (t=3.04, P=0.018). The ED_{50} for the decrease in attack bites after triazolam was lower in AHA mice than in ANA mice (9.16 μg/kg vs 11.40 μg/kg). Triazolam (30 μg/kg) also decreased tail rattles in ANA mice (t=3.99, P=0.001). Triazolam significantly lowered the frequency of grooming, rearing, and anogenital contact behavior in ANA mice, whereas this decrease was statistically significant only for walking in AHA mice.

Discussion

The current results highlight the important individual differences in the propensity to engage in heightened aggressive behavior after consuming moderate doses of alcohol, confirming previous studies in mice, rats and monkeys under various conditions (Winslow and Miczek 1985; Miczek et al. 1992, 1998). In the currently studied Swiss-Webster mice, a subgroup of individuals engaged in significantly higher rates of aggressive behavior after

alcohol self-administration. This result agrees with similar previous observations that involved experimenter-delivered and self-administered alcohol (Miczek et al. 1998; Miczek and de Almeida 2001). The current experimental findings extend these observations to measurements in an unfamiliar large locale for the confrontation, instead of the commonly used technique of confrontation in the resident's home cage. In unfamiliar surroundings, aggressive mice fight less than in their home cage in the absence of any treatment, and alcohol heightens this behavior significantly (Miczek and O'Donnell 1980). In the presently used large unfamiliar test cage, alcohol affected a broader range of the behavioral repertoire, and aggressive interactions were more distinctly organized in discrete bouts than in the smaller standard home cage for mice (Miczek et al. 1989).

The heightened aggressive behavior after self-administration of 1 g/kg alcohol was reduced by flumazenil and by β-CCt. However, the broad-spectrum benzodiazepine receptor antagonist flumazenil was about five times less potent and also less effective in reducing the alcohol-heightened aggressive behavior than β-CCt. The current flumazenil results on attenuating alcohol-heightened aggressive behavior in mice confirm similar findings in rats, monkeys and humans (Weerts et al. 1993; Bond et al. 1995; Weisman et al. 1998). The current data are the first to show that β-CCt, the antagonist with preferential action at $GABA_A$ receptors with α_1 subunits (Huang et al. 2000), reduces aggressive behavior in a dose-dependent manner, both in AHA and ANA mice. β-CCt was more potent and effective than flumazenil in reducing aggressive behavior in mice over the dose ranges tested, and this finding is closely similar to our results with rats (Gourley et al. 2002). However, β-CCt reversed neither the suppressive effects on aggressive behavior nor the sedative effects of higher doses of ethanol. It will be useful to confirm the present results with β-CCt using compounds that show a larger selectivity for $GABA_A$ receptors with α_1 subunits, preferably in gene-targeted point-mutated mice.

In alcohol-preferring rats, β-CCt reduces ethanol consumption (June et al. 2001; Harvey et al. 2002). Like β-CCt, 3-propoxy-β-carboline hydrochloride (3-PBC), a low-efficacy ligand with preferential action at receptors containing the α_1 subunit, suppresses alcohol-reinforced responding after central (i.e., anterior and medial ventral pallidum) and parenteral administrations. By contrast, flumazenil failed to reduce alcohol-reinforced responding after either parenteral or central injections (June et al. 1998). The failure to reduce the alcohol-motivated responding by flumazenil may be due to its partial agonist activity at some α_x β_3 γ_2 receptors. $GABA_A/\alpha_1$ receptors have been proposed to regulate the reinforcing effects of alcohol, at least in rats (Harvey et al. 2002). In the present study with outbred mice, flumazenil and β-CCt reduced alcohol-reinforced responding slightly or not at all. It appears that antagonists with either broad-spectrum or selective α_1 subunit activity can reduce alcohol-heightened aggressive behavior and the reinforcing effects of alcohol under some conditions. Whether or not compounds with selective action at other subtypes of the $GABA_A$ receptor may be more effective in reducing alcohol self-administration will have to await further study.

A distinctive feature of alcohol-heightened aggression is its occurrence in a subgroup of individuals (Miczek et al. 1998; de Almeida et al. 2001; Higley 2001; Miczek and de Almeida 2001), whereas most benzodiazepines like chlordiazepoxide, midazolam and diazepam and certain neurosteroids increase aggressive behavior in a broad range of individuals (Miczek 1974; Rodgers and Waters 1985; Weerts et al. 1993; Fish et al. 2001). The extent to which the ability to engage in alcohol-heightened aggressive behavior is an individual trait has not been established in animal models so far. The heritability of type-II alcoholism in humans, which is also characterized by the propensity to engage in fights but not necessarily under the influence of alcohol, would suggest the feasibility of a link between the vulnerability for alcohol drinking and aggressive behavior (Virkkunen and Linnoila 1993).

One intriguing observation from these studies is that not all benzodiazepine-type drugs increase aggressive behavior consistently (Bond and Lader 1988; Cherek et al. 1991; Kruk 1991; Martin-Lopez and Navarro 1996). For example, neither zolpidem nor triazolam enhanced aggressive behavior when tested alone (unpublished observations) or in the presence of alcohol. Instead, both compounds decreased aggressive behavior, an effect that may be attributed to the sedative effects of these drugs. By contrast, most classic benzodiazepines alter aggressive behavior in a biphasic fashion, with low doses increasing attacks and threats and high doses subsequently decreasing these behaviors (Miczek 1974; Miczek and Krsiak 1979; Rodgers and Waters 1985; Miczek et al. 1995, 2002).

The reason for differences among benzodiazepine-type compounds in modifying aggressive behavior is unclear, but may reflect distinct pharmacological properties related to action at $GABA_A$ receptors. For example, triazolam appears to have higher intrinsic efficacy, as measured by potentiation of GABA-induced chloride currents, than other benzodiazepines such as diazepam (Ducic et al. 1993). Triazolam is a highly effective hypnotic compared with other benzodiazepine agonists, and markedly impairs memory and motor activity (Kontinen et al. 1993; Ehrich et al. 1997). Zolpidem also is used to treat sleep disorders (Rush 1998) and has higher affinity for $GABA_A$ receptors containing α1 subunits than those with other subunit compositions (Cox et al. 1995). Many of the characteristic behavioral effects of zolpidem have been attributed to its binding to the $GABA_A/\alpha_1$ receptor (Crestani et al. 2000; Rowlett et al. 2003). Altogether, these findings raise the possibility that the degree of intrinsic efficacy and/or selectivity for $GABA_A$ receptor subtypes might be a determinant of a drug's ability to increase aggressive behavior. In particular, these results suggest that stimulation of the $GABA_A/\alpha_1$ receptor subtype may play a key role in mediating aggression that is enhanced by alcohol.

Acknowledgements We acknowledge support by USPHS research grants AA13983, DA02632, DA11792, and grants from the Alcoholic Beverage Medical Research Foundation. We are grateful to Mr. J. Thomas Sopko, Mr. Daaniel Heerewijn and Ms. Sara Faccidomo who provided outstanding support.

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