Behavioral Characterization of the Novel GABAB Receptor-Positive Modulator GS39783 (N,N′-Dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine): Anxiolytic-Like Activity without Side Effects Associated with Baclofen or Benzodiazepines

John F. Cryan, Peter H. Kelly, Frederique Chaperon, Conrad Gentsch, Cedric Mombereau,[1] Kurt Lingenhoehl, Wolfgang Froestl, Bernhard Bettler,[2] Klemens Kaupmann, and Will P. J. M. Spooren[3]

Neuroscience Research, Novartis Institutes for BioMedical Research, Novartis Pharma AG, Basel, Switzerland

ABSTRACT

The role of GABAB receptors in various behavioral processes has been largely defined using the prototypical GABAB receptor agonist baclofen. However, baclofen induces sedation, hypothermia and muscle relaxation, which may interfere with its use in behavioral paradigms. Although there is much evidence for a role of the inhibitory neurotransmitter GABA in the pathophysiology of anxiety, the role of GABAB receptors in these disorders is largely unclear. We recently identified GS39783 (N,N′-dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine) as a selective allosteric positive modulator at GABAB receptors. The aim of the present study was to broadly characterize the effects of GS39783 in well-validated rodent models for motor activity, cognition, and anxiety. The following tests were included: locomotor activity in rats and mice, rotarod and traction tests (including determinations of core temperature) in mice, passive avoidance in mice and rats, elevated plus maze in rats, elevated zero maze in mice and rats, stress-induced hyperthermia in mice, and pentobarbital- and ethanol-induced sleep in mice. Unlike baclofen and/or the benzodiazepine chlordiazepoxide, GS39783 had no effect in any of the tests for locomotion, cognition, temperature, or narcosis. Most interestingly, GS39783 had anxiolytic-like effects in all the tests used. Overall, the data obtained here suggest that positive modulation of GABAB receptors may serve as a novel therapeutic strategy for the development of anxiolytics, with a superior side effect profile to both baclofen and benzodiazepines.

GABA is the main inhibitory neurotransmitter in the brain; hence, GABAergic neurotransmission regulates many physiological and psychological processes. There are two classes of GABA receptors: ionotropic GABA receptors, including GABAA and GABAC receptors, and metabotropic GABAB receptors (Barnard et al., 1998; Bormann, 2000; Bowery et al., 2002). The GABAB receptor is a heterodimer made up of two subunits, GABAB(1) and GABAB(2), both necessary for GABAB receptors to be functionally active (Calver et al., 2002). Pharmacological studies with baclofen, the prototypical highly selective GABAB receptor agonist, have pointed to a role for GABAB receptors in epilepsy, cognition, pain, gastroesophageal reflux disease, and addiction (Bowery et al., 2002). Baclofen has been in clinical use for the treatment of spasticity for over 30 years (Brogden et al., 1974). However, it is this muscle relaxing property, together with the compound's sedative and hypothermic effects, which limit its widespread use as a tool in behavioral pharmacological studies.

Allosteric, positive modulation of metabotropic receptors is

ABBREVIATIONS: [35S]GTPγS, guanosine 5′-O-(thio)triphosphate; GS39783, N,N′-dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine; CDZ, chlordiazepoxide; ANOVA, analysis of variance; CGP55845A, [3-[[1-(S)-(3,4-dichlorophenyl)ethyl]amino]-2-(S)-hydroxy-propyl]phenyl-methyl-phosphinic acid hydrochloride; SIH, stress-induced hyperthermia.

a newly identified phenomenon, providing novel means for the pharmacological manipulation of G-protein-coupled receptors acting at a site apart from the orthosteric binding region of the receptor protein (Soudijn et al., 2002; Jensen and Spalding, 2004). Allosteric modulators are therefore thought to offer a number of potential pharmacological improvements when compared with the use of conventional agonists, as has been demonstrated for modulators acting at ligand-gated ion channels (Costa, 1989). Modulators at the GABA_A receptors are used therapeutically; for example, benzodiazepines amplify the action of the endogenous neurotransmitter GABA at the GABA_A receptor. More recently, novel positive allosteric modulators of the GABA_B receptor have been identified (Urwyler et al., 2001, 2003). In analogy to the effects of benzodiazepines on GABA_A receptors, we hypothesize that GABA_B receptor-positive modulators might represent therapeutically superior drugs compared with full GABA_B receptor agonists with respect to undesired side effects.

The GABA_B receptor-positive modulator GS39783 has of late been characterized in vitro (Urwyler et al., 2003). GS39783 potentiates both the potency and maximal efficacy of GABA-stimulated guanosine $5'$-O-(thio)triphosphate ([^{35}S]GTPγS) binding to membranes from a GABA_{B(1b/2)}-expressing Chinese hamster ovary cell line but does not stimulate [^{35}S]GTPγS binding itself. Potentiation of GABA_B receptor responses by GS39783 is also observed using native GABA_B receptor preparations from rat brain. Furthermore, like baclofen, GS39783 suppresses paired pulse inhibition in rat hippocampal slices. This effect is reversed by the competitive GABA_B receptor antagonist CGP55845A and is produced most likely by enhancing the effect of synaptically released GABA at presynaptic GABA_B receptors. In functional GTPγS binding assays, positive modulation by GS39783 was observed to be GABA_B receptor selective because functional responses from a related receptor, the mGluR2, were not enhanced by the compound (Urwyler et al., 2003). Furthermore, 1 μM GS39783 was inactive in binding assays on more than 30 different receptor proteins (G-protein-coupled receptors, transporters, and ion channels (N. Subramanian, unpublished data).

Although GABAergic neurotransmission is long known to play a critical role in anxiety, data on the specific role of GABA_B receptors in anxiety are limited and rather variable (see Millan, 2003). This is largely because investigators relied on using baclofen for such an analysis, a compound having only a narrow efficacy window before confounding side effects are manifested in anxiety paradigms (Dalvi and Rodgers, 1996). Renewed interest in the role of GABA_B receptor in anxiety has recently emerged; GABA_{B(1)}-deficient mice were found to be more anxious than their wild-type counterparts (Mombereau et al., 2004). With the availability of GS39783, we now have a new tool to assess the role of GABA_B in anxiety disorders. In the present studies, we broadly characterized GS39783 in behavioral paradigms where full agonists, such as baclofen, have been shown to induce alterations; these include models of motor performance, cognition, and body temperature. Furthermore, we characterize the effects of GS39783 in a number of well-validated animal models of anxiety and compare its efficacy and side effect profile with that of the benzodiazepine chlordiazepoxide (CDZ).

Materials and Methods

Animals

All animals were purchased from Iffa Credo (L'Arbresle, France). The animals had access to water and food ad libitum and were experimentally naive. Rats and mice were separately housed in macrolon cages ($42 \times 26 \times 15$ cm or $55 \times 33 \times 19$ cm; $n = 4$ per cage for rats; $n = 10$–15 per cage for mice) in a temperature-controlled room under artificial illumination. Lights were maintained on a 12-h light/dark cycle (lights on 6:00–6:30 AM depending on experiment). Male OF1/IC mice (18–35 g) were used in all mouse studies with the exception of the passive avoidance test where male CD-1 mice (25–35 g) were used and the rotarod ethanol study where BALB/c mice (25–30 g) were used. Male Sprague-Dawley rats (160–180 g) were used for locomotor activity, and elevated plus maze larger animals (250–300 g) were used for passive avoidance, whereas male Wistar rats (180–200 g) were used for elevated zero maze. All studies were performed according to methods approved by the Veterinary Authority of the City of Basel.

Drugs

All drugs were made up fresh prior to use. For all studies, GS39783 (Novartis, Basel, Switzerland), l-baclofen (Novartis), and chlordiazepoxide hydrochloride (Sigma-Aldrich, St. Louis, MO) were dissolved in 0.5% methylcellulose (vehicle) or in distilled water with a few drops of Tween 80 (elevated zero maze) solution as a fine suspension (GS39783). They were applied p.o. in a volume of 1 ml/kg to rats and 10 ml/kg to mice unless otherwise noted.

Statistics

A Kruskal-Wallis analysis of variance (ANOVA) was used followed by the Mann-Whitney U test (Bonferroni corrected) for rotarod test. The Fisher's exact test was used (Bonferroni corrected) for the traction test. As appropriate, a repeated or single ANOVA was used followed by either Dunnett's or Fisher's post hoc test for locomotor activity, temperature, passive avoidance, elevated zero maze, elevated plus maze, stress-induced hyperthermia, and ethanol-induced rotarod impairment. For rat locomotor activity, plus maze and zero maze, the effects of comparator compound (baclofen or chlordiazepoxide) were against control was compared using a Student's t test.

Effects of GS39783 on Rotarod and Traction Test

Rotarod Test. The rotarod apparatus consisted of a cylinder subdivided into five available mice positions, each 6 cm in diameter, which was positioned 30 cm above the table and rotated at a speed of 12 rpm (Dunham and Miya, 1957). The mice were placed singly on the cylinder. On the day before the start of the experiment, animals were trained to stay on the rotarod for 300 s. Mice that failed to learn the test or did not reach the criterion (300-s endurance) were excluded from the study. During the test day, the length of time each mouse remained on the cylinder (="endurance time," maximal score 300 s) was measured 1, 3, 6, and 24 h after application of a test compound or vehicle.

Traction. The animal was suspended by the front paws from a horizontal wire. The test was successfully completed when the animal was able to touch the wire with at least one hind paw within 5 s (Boissier and Simon, 1960). The animals were tested 1, 3, 6, and 24 h after application immediately prior to the rotarod test (see above).

Treatments. Mice received oral administrations of GS39783 (0.1, 1, 10, 50, 100, or 200 mg/kg p.o.), l-baclofen (2.5 or 5 mg/kg p.o.), chlordiazepoxide (5 or 10 mg/kg p.o.), or vehicle (0.5% methylcellulose p.o.).

Effects of GS39783 on Core Body Temperature

Body Temperature. Rectal temperature was measured to the nearest 0.1°C by an ELLAB instruments thermometer (Copenhagen, Denmark) via a lubricated thermistor probe (2-mm diameter) in-

serted 20 mm into the rectum while the mouse was hand held near the base of the tail. The probe was left in place until steady readings were obtained (approximately 15 s). The animals were tested −1, 0, 1, 2, and 4 h after application with a test compound or vehicle.

Treatments. Mice received oral administrations of GS39783 (25, 50, 100, or 200 mg/kg p.o.), *l*-baclofen (5, 10, or 15 mg/kg p.o.), or vehicle (0.5% methylcellulose p.o.).

Effects of GS39783 on Locomotor Activity

Rats. *Recording device.* Horizontal locomotor activity was assessed in transparent Plexiglas boxes (dimensions: 19 × 31 × 16 cm), and activity was detected and registered using the TSE Moti system (TSE, Bad Homburg, Germany), which is based on the registration of infrared light beam interruptions along the *x*-, *y*-, and *z*-axes, as caused by an animal's movements (Spooren et al., 2000); data were directly stored into the computer.

Experimental procedures. Rats received injections with GS39783 (doses 0.1, 1, 10, 100, and 200 mg/kg p.o.), *l*-baclofen (5 mg/kg p.o.), or vehicle (0.5% methylcellulose p.o.) and were immediately returned to their home cages for 60 min.

Mice. *Recording device.* See above for rats.

Experimental procedures. Mice received injections with GS39783 (doses 1, 3, 10, 30, and 100 mg/kg p.o.), baclofen (1, 3, and 10 mg/kg p.o.), or vehicle (0.5% methylcellulose p.o.) and also were immediately returned to their home cages for 60 min. Subsequently, they were individually placed in Plexiglas boxes (as for rats), and their spontaneous locomotor activity was registered for the next 30 min.

Effects of GS39783, Baclofen, and Chlordiazepoxide on Passive Avoidance Behavior

Mice. *Experimental procedure.* Starting on the day before the training trial and continuing throughout the experiment, they were housed singly in the experimental room. The apparatus and procedure were as previously described in detail (Venable and Kelly, 1990). Briefly, for the training trial, mice were gently placed into the light side of the two-compartment trough-shaped apparatus. The door to the dark compartment was opened and a button pressed to initiate timing by the computer. When the mouse broke a photocell beam located 10.5 cm into the dark compartment, the latency from opening the door to the animal breaking the beam (step-through latency) was automatically recorded, and a Campden Instruments 521 C Shock Source (Campden Instruments Ltd., Leicester, UK) was automatically activated. This resulted in the application of a foot shock (0.5-mA rectangular current waves) between the stainless steel plates, which comprised the dark compartment. This ended when the mouse escaped back to the light compartment or after 5 s elapsed, whichever came first. If an animal did not enter the dark compartment within 150 s on the training trial, it was removed from the apparatus without receiving any shock and was excluded from the retention test. The retention test was carried out on the following day, and the same procedure was followed except that the shock generator was switched off.

Drugs. GS39783 (1, 3, 10, 30, and 100 mg/kg) or chlordiazepoxide (5, 10, 20, and 40 mg/kg) were applied p.o. 60 min before the training trial. *l*-Baclofen (1 and 3 mg/kg) was administered s.c. 30 min before the training trial.

Rats. Since more pronounced effects of GS39783 emerged in the rat version of the elevated zero maze compared with mouse test, we also sought to clarify if undesired effects would also emerge in rats even though none were present in mice. Therefore, we tested the effects of GS39783 on passive avoidance.

Experimental procedure. On the day before the experiment, rats were housed singly in the experimental room and were handled twice for 30 to 60 s each. The passive avoidance apparatus for rats was trough shaped like that for mice (see above) and was exactly as previously described (Venable and Kelly, 1990).

Drugs. GS39783 (25, 50, and 100 mg/kg), *l*-baclofen (1 and 3

mg/kg), and chlordiazepoxide (20 and 40 mg/kg) or vehicle (0.5% methylcellulose) were used.

The Effects of GS39783 on Behavior in the Elevated Zero Maze Test

Mice. *Apparatus.* The apparatus was a 5.5-cm-wide circular track constructed of gray Plexiglas with an inside diameter of 34 cm, a midtrack circumference of approximately 121 cm, and an elevation of 40 cm. It consisted of two open quadrants with a raised, 2-mm edge and two closed quadrants with walls 11 cm high. Because the zero maze had no central area, the animal had to be in either an open or a closed part of the arena.

Experimental procedure. After oral drug administration (60 min prior to test), the animals were returned to their home cage. Mice were placed in one of the closed quadrants designated as the starting quadrant and were allowed to investigate the zero maze for a period of 5 min. During this time, an observer scored mice on several anxiety-related variables as identified in previous studies (Shepherd et al., 1994; Tarantino et al., 2000). These included time spent in both open and closed quadrants, number of transitions between quadrants, latency to leave the closed quadrant, stretchings (elongated body posture with at least snout over open/closed divide) into open quadrant, rearings, and head dips.

Drugs. GS39783 (3, 10, and 30 mg/kg p.o.), chlordiazepoxide (10 mg/kg p.o.), or (vehicle 0.5% methylcellulose) were administered 60 min before the test.

Rats. *Apparatus.* The maze was essentially a larger version of the mouse test and consisted of a gray Plexiglas annular platform (105-cm diameter, 10-cm width) elevated to 65 cm above floor and divided equally into four quadrants. Two opposite quadrants were enclosed by gray Plexiglas walls (height: 27 cm for the outer wall and 20 cm for the inner wall), whereas the remaining two opposite quadrants were surrounded by a small border (1 cm high). The apparatus was illuminated by white light (150–190 lux).

Experimental procedure. After oral drug administration, the animals were returned to their home cage. After an appropriate pretreatment time (60 min), rats were individually placed into a closed section. A 5-min trial was performed, and between subjects, the maze was thoroughly cleaned with Thedra (Thedra, Zwingen, Switzerland). Unlike the mouse test, the different parameters [the time spent in open quadrants, the distance ratio (expressed as the distance traveled in open quadrants/total distance traveled), and the total distance traveled] were automatically recorded and analyzed by means of an in-house developed video-tracking system and quantified with appropriate software. Briefly, an ordinary black and white camera mounted 2 m above the zero maze was connected to a frame grabber (type DT3155; Data Translation, Inc., Marlboro, MA). Every second, the digitized frame was compared with the previously stored frame, whereby the pixels with altered intensity were identified and used to compute the position of the animal and the distance traveled. In addition, the number of stretched attend postures (elongated body posture with at least snout over open/closed divide) into the open quadrants and the number of head dips were scored manually by an experimenter sitting in the room. Trials were performed between 11:30 AM and 4:30 PM. Unlike the mouse version of the test, the latency to exit the closed quadrant was not analyzed due to high variability of this parameter in rats (F. Chaperon, unpublished data).

Drugs. Rats were treated with GS39783 (3, 10, and 30 mg/kg p.o.), chlordiazepoxide-HCl (10 mg/kg p.o.), or vehicle (distilled water with 1% Tween 80) 60 min before the test in a volume of 2 ml/kg.

Effects of GS39783 in the Elevated Plus Maze Test of Anxiety

Apparatus. The elevated plus maze consisted of two open arms (40 × 12 cm) and two enclosed arms (40 × 12 × 20 cm) that all extended from a common central platform (12 × 12 cm). The config-

TABLE 1
Effects of GS39783, baclofen, and chlordiazepoxide on motor performance in the rotarod and traction test

Effects of GS39783 (0, 1, 3, 10, and 30 mg/kg p.o.), l-baclofen (2.5–5 mg/kg), and chlordiazepoxide (10 mg/kg p.o.) on rotarod endurance traction and core body temperature in mice. Values represent mean and S.E.M. $n = 10$ per treatment group.
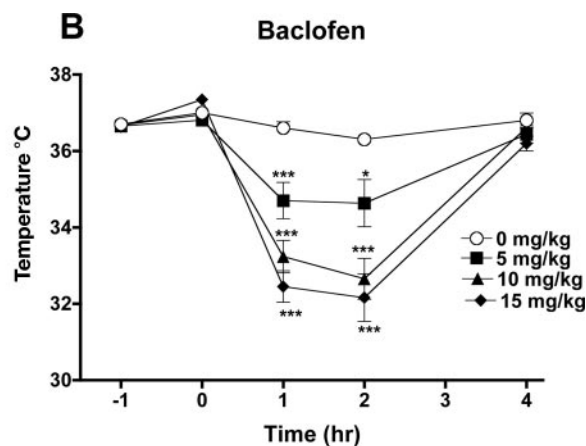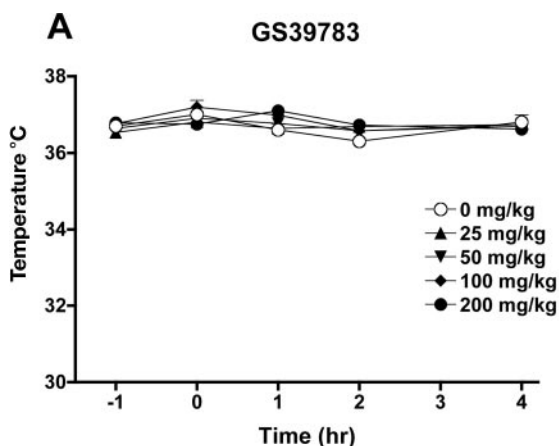
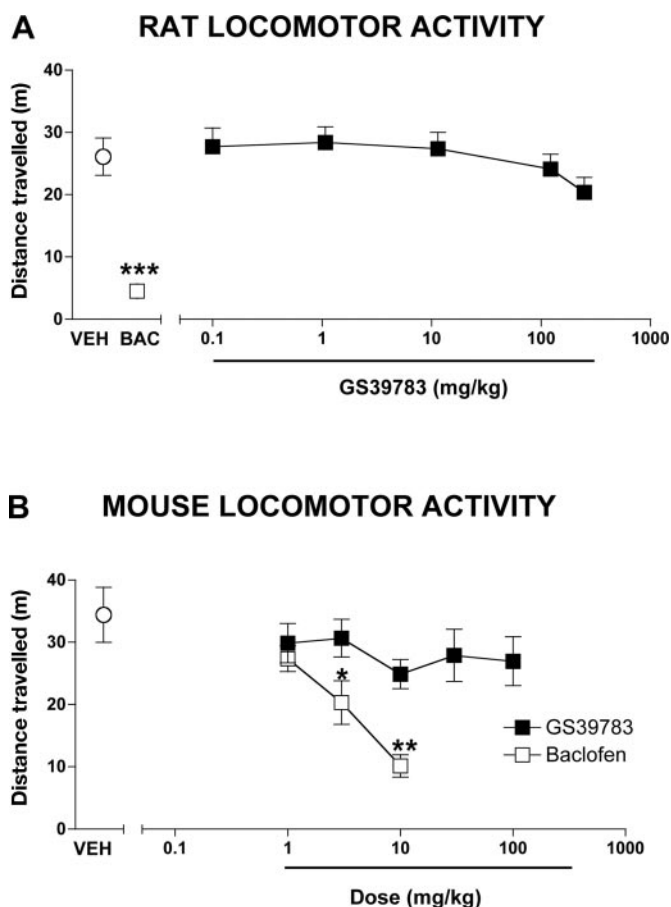| Drug	Rotarod Endurance	Traction Test
	s	*% Successful*
Vehicle	300 + 0	100 + 0
GS39763		
0.1 mg/kg	300 + 0	
1 mg/kg	300 + 0	100 + 0
10 mg/kg	300 + 0	100 + 0
50 mg/kg	300 + 0	100 + 0
100 mg/kg	300 + 0	100 + 0
200 mg/kg	231 + 37	100 + 0
Baclofen		
2.5 mg/kg	214 + 38	50*
5 mg/kg	171 + 43*	30**
Chlordiazepoxide		
5 mg/kg	208 + 34	100 + 0
10 mg/kg	236 + 33	100 + 0

* $p < 0.05$.
** $p < 0.01$ vs. vehicle-treated mice.

uration formed the shape of a plus sign, with similar arms arranged opposite to one another, and the apparatus was elevated 60 cm above the floor on a central pedestal. The maze was made from gray Plexiglas. The grip on the open arms was facilitated by inclusion of a small raised edge (0.25 cm) around their perimeter.

Drug Treatment and Experimental Procedure. The method was adopted from Handley and Mithani (1984). Rats were randomly allocated to one of the treatments. Animals were transported from the housing room to the laboratory at least 1 h before testing. Following oral drug administration, rats were individually housed in macrolon cages (22 × 16 × 14 cm) and after 60 min placed onto the central platform facing an enclosed arm. An 8-min trial was performed, and between subjects, the maze was thoroughly cleaned with Thedra. Direct registrations were made by an observer sitting close to the maze, and the following conventional parameters were used: number of open and closed arm entries (arm entry defined as all four paws entering an arm) and time spent on open arms (excluding the central platform), distance traveled on open arms, grooming time, and the number of rearings. Additionally, the ratio of open/total arm entries was calculated, which takes into account any potential confounding motor effects of compounds. Animals from the different treatment-groups were alternatively tested, and trials were performed between 8:30 AM and 12:30 PM, i.e., within the first half of the light phase.

Fig. 2. The effects of GS39783 and baclofen on locomotor activity in rats and mice. A, effect of acute GABA$_B$ positive modulators (0.1, 1, 10, 100, and 200 mg/kg p.o) and l-baclofen (BAC) (5 mg/kg p.o.) on locomotor activity in rats ($n = 15$–16) compared with vehicle (VEH)-treated animals. B, effect of acute GABA$_B$-positive modulators (1, 3, 10, 30, and 100 mg/kg p.o) and l-baclofen (1, 3, and 10 mg/kg p.o) on locomotor activity in mice ($n = 8$) compared with vehicle (VEH)-treated animals. Values are means ± S.E.M. *, **, and ***, groups that differed significantly from vehicle-treated animals ($p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively).

Drug Treatment. Rats were treated with GS39783 (doses: 0.1, 1, 10, or 100 mg/kg p.o.), chlordiazepoxide (10 mg/kg p.o.), or vehicle (0.5% methylcellulose) ($n = 15$ per group).

Fig. 1. The effects of GS39783 and baclofen on core body temperature of mice. A, effect of acute GABA$_B$ positive modulators (25, 10, 100, and 200 mg/kg p.o). B, l-baclofen (5, 10, and 15 mg/kg p.o.) on core body temperature in mice ($n = 12$). Values are means ± S.E.M. * and **, groups that differed significantly from vehicle-treated animals ($p < 0.05$, and $p < 0.001$, respectively).

### The Effects of GS39783 on Stress-Induced Hyperthermia Test of Anxiety

Experimental Procedure. Mice were singly housed in smaller macrolon cages (26 × 21 × 14 cm) 24 h before testing. The test procedure for the modified stress-induced hyperthermia was adopted from Van der Heyden et al. (1997) and was based on the original description of stress-induced hyperthermia by Lecci et al. (1990). Rectal temperature was measured to the nearest 0.1°C by an ELLAB instruments thermometer via a lubricated thermistor probe (2-mm diameter) inserted 20 mm into the rectum while the mouse was hand held near the base of the tail. The probe was left in place until steady readings were obtained (approximately 15 s). Stress-induced hyperthermia was assessed as follows: The core-temperature of each mouse was measured twice. The second measurement was 15 min after the first measurement, which served as the basal value for each condition. The depen-

dent variable, i.e., the stress-induced hyperthermia, was defined as the difference between the second measurement and the first measurement. The first measurement was used to evaluate whether the test compound by itself would have a potential effect on basal body temperature.

Drug Treatment. Sixty minutes before measuring the first measurement, the animals received an oral administration of GS39783 (doses 0.01, 0.1, 1 3, 10, or 30 mg/kg) or vehicle (0.5% methylcellulose).

The Effects of GS39783 on Ethanol-Induced Sedation and Narcosis and on Pentobarbital-Induced Narcosis

Rotarod Studies. *Experimental procedures.* The rotarod test was carried out as described in experiment 1. During the test day, the length of time each mouse remained on the cylinder (=endurance
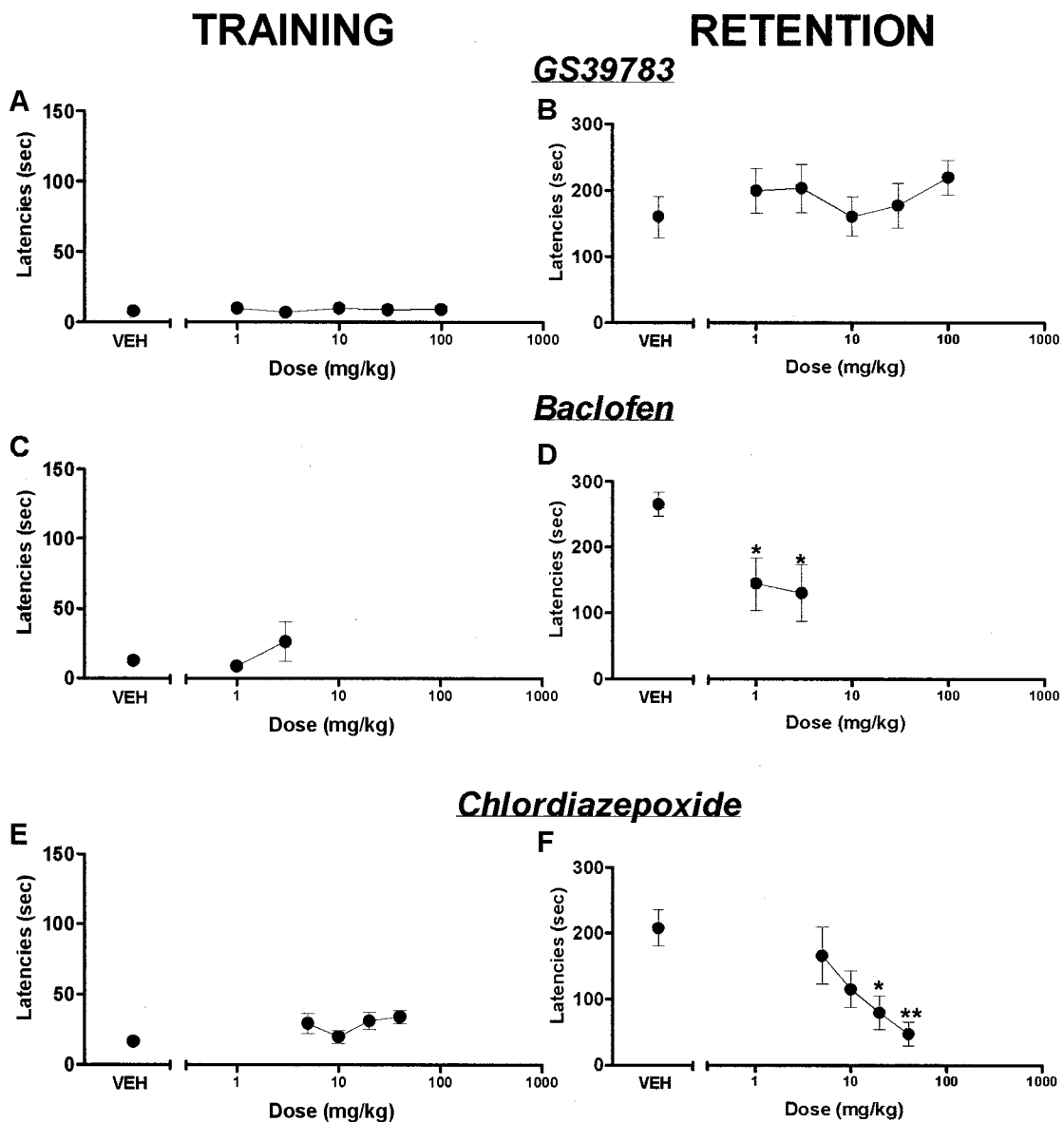
Fig. 3. The effects of GS39783, baclofen, and chlordiazepoxide on passive avoidance behavior in mice. GABA$_B$-positive modulator GS39783 (1, 3, 10, 30, and 100 mg/kg p.o.) did not affect latencies to step through into the dark during training (A) and retention (B) sessions compared with vehicle (VEH)-treated animals. Conversely, *l*-baclofen (1 and 3 mg/kg s.c.) and chlordiazepoxide (20 and 40 mg/kg p.o.) treatment induced a decrease of latencies to step through into the dark during retention session (D and F, respectively) compared with vehicle (VEH)-treated animals but not in training session (C and E, respectively) compared with vehicle (VEH)-treated animals. GS39783 experiment: n = 10 per treatment group. Baclofen experiment: n = 10 per treatment group. Chlordiazepoxide experiment: n = 8 per treatment group. All values represent mean values, with vertical lines indicating one S.E.M. * and **, groups that differed significantly from vehicle-treated mice ($p < 0.05$ and $p < 0.01$, respectively).

time, maximal score 300 s) was measured 1, 2, and 4 h after application of GS39783 or vehicle.

Treatments. Mice received oral administrations of GS39783 (1, 3, 10, and 30 mg/kg p.o.), chlordiazepoxide (10 mg/kg p.o.), or vehicle (0.5% methylcellulose p.o.). Thirty minutes later, mice received an injection of ethanol (20%) or vehicle. The dose of ethanol was chosen based on dose-finding studies showing that this concentration had threshold effects on motor impairment (J. F. Cryan, unpublished data).

Narcosis Studies. *Experimental procedure.* Sleep was induced by either 50 mg/kg i.p. pentobarbital or by 10 ml/kg i.p. of a 40% ethanol-solution (4 g/kg) and quantified by assessing the period between the loss of the righting reflex and its return. A cutoff at 150 min was applied, and those mice that did not fall asleep at all or died (maximally $n = 1$ per group in the present study) were excluded. Doses of pentobarbital and ethanol were chosen based on previous dose-finding studies (C. Gentsch, unpublished data), which demonstrated that these doses caused a reliable but submaximal sleep to allow for prolongations of the duration of sleep by test compounds within the 150-s cutoff period.

Pentobarbital-induced sleeping time. Two separate studies were performed; groups of $n = 15$ mice were orally pretreated with 1, 10, or 100 mg/kg or 0.3, 3, or 30 mg/kg p.o. GS39783, respectively. In addition to the vehicle-treated group (0.5% methylcellulose), an additional group was pretreated, per experiment, with 7.5 mg/kg chlordiazepoxide and served as "positive" standard. Sixty minutes later, all mice were treated intraperitoneally with 50 mg/kg pentobarbital (Fluka, Buchs, Switzerland).

Ethanol-induced sleeping time. Groups of $n = 15$ mice were orally pretreated with vehicle (0.5% methylcellulose) or with 1, 10, or 100 mg/kg p.o. GS39783 or 7.5 mg/kg chlordiazepoxide. Sixty minutes later, all mice were treated intraperitoneally with 4 g/kg ethanol (Merck, Darmstadt, Germany).

Results

Effects of GS39783 on Rotarod and Traction Test

Rotarod Test. *GS39783.* ANOVA indicated a significance 1 h after application only ($p < 0.01$). However, post hoc tests found no significance at any dose level (Table 1).

l-*Baclofen.* ANOVA indicated a significance 1 h after application only. The post hoc test indicated that endurance performance was significantly reduced at the dose of 5 mg/kg l-baclofen (Table 1).

Chlordiazepoxide. The ANOVA revealed no significant effect ($p = 0.056$), but 1 h after application, rotarod endurance

was significantly reduced at the dose of 5 mg/kg as compared with vehicle (Table 1).

Traction. *GS39783.* All animals treated with any dose of GS39783 (0.1–200 mg/kg p.o.) were successful in the traction test at all time points (Table 1).

l-*Baclofen.* The number of successful animals in the traction test was significantly reduced at 1 h after application of 2.5 and 5 mg/kg ($p < 0.5$ and $p < 0.01$, respectively).

Chlordiazepoxide. Following both 5 and 10 mg/kg p.o. chlordiazepoxide, all animals were successful in the traction test at all time points (Table 1).

There were no significant effects of any drug at other time points tested (data not shown).

Effects of GS39783 on Core Body Temperature

Repeated measures ANOVA revealed a significant effect of drug [$F(7,88) = 27.10$, $p < 0.001$], a significant effect of time [$F(4,352) = 52.97$, $p < 0.001$], and a significant drug treatment $\times$ time interaction [$F(28,352) = 25.90$, $p < 0.001$]. Post hoc analysis revealed that all doses of baclofen (5–15 mg/kg) significantly lowered core body temperature 1 and 2 h following administration, whereas GS39783 did not modify temperature at any time point tested. The effects of baclofen on temperature had dissipated 4 h following injection (see Fig. 1).

Effects of GS39783 on Locomotor Activity

Rats. GS39783 at doses of 0.1, 1, 10, 100, or 200 mg/kg had no effect on spontaneous locomotor activity in rats. In contrast, a highly significant effect of l-baclofen (5 mg/kg p.o.) on activity ($p < 0.001$) was found (see Fig. 2A).

Mice. ANOVA revealed a significant effect of drug treatment on locomotor activity in mice [$F(8,63) = 4.31$, $p = 0.001$]. Post hoc revealed that GS39783 did not affect the locomotor activity (all doses tested). In contrast, l-baclofen induced a marked hypolocomotion at 3 and 10 mg/kg ($p < 0.01$ and $p < 0.001$, respectively) (see Fig. 2B).

Effects of GS39783 and Baclofen on Passive Avoidance Behavior

Mice. When mice received GS39783 (1–100 mg/kg) orally 60 min before the training trial, it had no effect on latencies to step through into the dark (shock) compartment on either the training trial or retention test. In all groups, a high
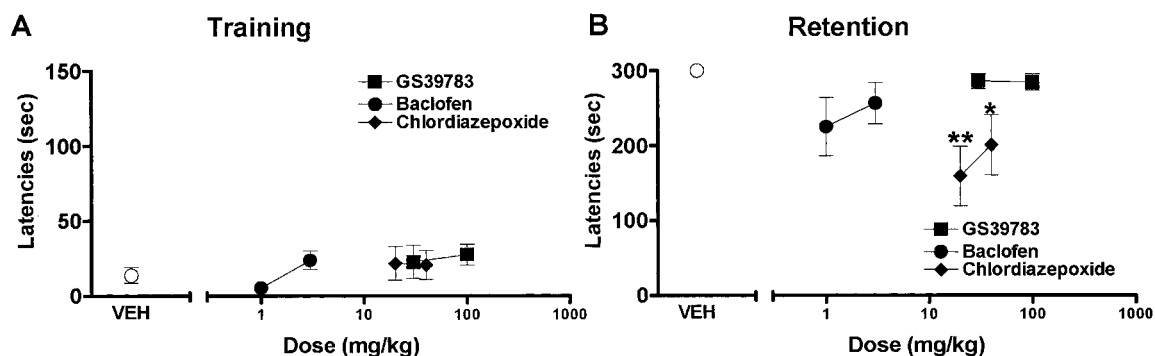
Fig. 4. The effects of GS39783, baclofen, and CDZ on passive avoidance behavior in rats. The GABA_B-positive modulator GS39783 (10, 30, and 100 mg/kg p.o.) and l-baclofen (1 and 3 mg/kg p.o.) did not affect latencies to step through into the dark during training (A) and retention (B) sessions compared with vehicle (VEH)-treated animals. However, CDZ (20 and 40 mg/kg p.o.) treatment induced a decrease of latencies to step through into the dark during retention session (A) but not in training session (B) compared with vehicle (VEH)-treated animals. ($n = 7$–11). * and **, groups that differed significantly from vehicle-treated mice ($p < 0.05$ and $p < 0.01$, respectively).

degree of learning was produced as indicated by prolonged step-through latencies in the retention test. In contrast, chlordiazepoxide, although not affecting latencies in the training trial, significantly reduced latencies in the retention test [$F(4,35) = 4.80$, $p = 0.003$], with both the 20 and the 40 mg/kg groups differing significantly from the vehicle group ($p < 0.05$ and $p < 0.01$, respectively, two-tailed Dunnett's test). In the l-baclofen experiment, one animal did not enter the shock compartment within the 150-s limit and was therefore excluded from the retention test. Like chlordiazepoxide, baclofen did not affect latencies in the training trial but significantly reduced latencies in the retention test [$F(2,26) = 4.80$, $p = 0.003$], with both the 20 and the 40 mg/kg groups differing significantly from the vehicle group ($p < 0.05$ and $p < 0.01$, respectively) (see Fig. 3).

Rats. Two animals (one from the 3 mg/kg baclofen group and one from the 40 mg/kg chlordiazepoxide group) failed to enter the dark compartment within the 150-s limit on the training day and were excluded from the experiment. One-way ANOVA of training latencies showed no significant effect of treatment group. However, there was a significant effect of treatment group [$F(6,59) = 4.04$, $p = 0.002$] on retention test latencies, with both chlordiazepoxide groups showing shorter latencies than the control group ($p < 0.05$ and $p < 0.01$ for the 20 and 40 mg/kg groups, respectively) (Fig. 4).

The Effects of GS39783 on Behavior in the Elevated Zero Maze Test of Anxiety

Mice. *Latency.* The ANOVA indicated that a significant effect of treatment [$F(5,63) = 2.82$, $p = 0.023$] (Fig. 5A). Post hoc revealed a decrease of latency to enter in open quadrants only in chlordiazepoxide (10 mg/kg)-treated mice (Fig. 5A).

Time spent in open quadrants. As shown in Fig. 4B, ANOVA [$F(5,63) = 10.36$, $p < 0.001$] revealed that treatment with chlordiazepoxide (10 mg/kg) increased time spent in open quadrant. In contrast, GS39783 had no effect on this parameter (Fig. 5B).

Number of line crossings. ANOVA revealed a significant effect of dug treatment on the number of line crossings during the test [$F(5,63) = 19.79$, $p < 0.001$]. Only chlordiazepoxide (10 mg/kg), not GS39783, increased this parameter ($p < 0.001$) (data not shown).

Number of stretched attend postures. As indicated by the ANOVA [$F(5,63) = 13.89$; $p < 0.001$], treatment with GS39783 induced an overall effect on number of stretched attend postures (Fig. 5C). Post hoc tests revealed that GS39783 at 10 and 30 mg/kg significantly decreased the number of stretched attend postures ($p = 0.004$ and $p = 0.001$). Chlordiazepoxide (10 mg/kg) also significantly decreased this parameter ($p < 0.001$).

Number of head dips. ANOVA indicated an intergroup difference for the number of head dips [$F(5,63) = 5.622$, $p < 0.01$]. Post hoc comparisons revealed that GS39783 had no effect on the number of head dips. Chlordiazepoxide (10 mg/kg) induced a significant increase in the number of head dips ($p < 0.001$) (data not shown).

Number of rearings. The ANOVA indicated that treatment significantly modified the number of rearings [$F(5,63) = 4.162$, $p = 0.002$]. Post hoc tests revealed that GS39783 at 30 mg/kg significantly increased the number of rearing ($p = 0.024$). Chlordiazepoxide (10 mg/kg) also significantly increased this parameter ($p < 0.001$) (data not shown).

Rats. *Time spent in open quadrants.* As shown in Fig. 6A, ANOVA [$F(3,36) = 5.09$, $p < 0.01$] revealed that treatment with GS39783 induced an overall effect on the time spent in open quadrants. Subsequent analysis indicated that all tested doses of GS39783 (3, 10, and 30 mg/kg) produced a significant increase in the time spent in open quadrants ($p < 0.001$ and $p < 0.01$). As expected for a positive standard, chlordiazepoxide (10 mg/kg) also significantly increased the time spent in open quadrants ($p < 0.01$).

Total distance traveled. ANOVA revealed no significant effect of GS39783 on the total distance traveled during the
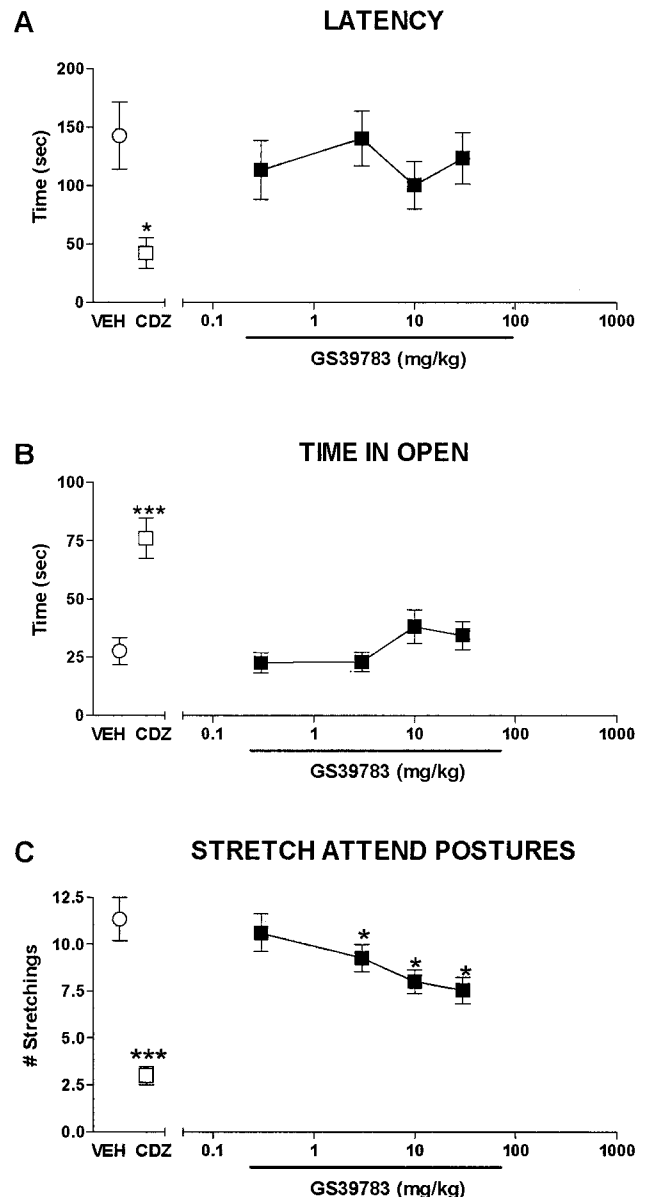
Fig. 5. The effects of GS39783 and CDZ on behavior in the mouse elevated zero maze. A, latency. Only CDZ significantly decreased the latency to enter into the open quadrants compared with vehicle (VEH)-treated animals. B, time in open. Only CDZ significantly decreased the latency to enter into the open quadrants compared with vehicle (VEH)-treated animals. C, stretched attend postures. Both the GABA$_B$-positive modulator GS39783 and chlordiazepoxide (10 mg/kg p.o.) decreased the number of stretched attend postures compared with vehicle (VEH)-treated animals. $n = 12$ per treatment group. All values represent mean values, with vertical lines indicating one S.E.M. ∗∗ and ∗∗∗, groups that differed significantly from vehicle-treated mice ($p < 0.01$ and $p < 0.001$, respectively).

test. In contrast, chlordiazepoxide (10 mg/kg) significantly increased this parameter ($p < 0.01$) (Fig. 6D).

Distance ratio. As indicated by the ANOVA [$F(3,36) = 3.02$, $p < 0.05$], treatment with GS39783 induced an overall effect on the distance ratio. Post hoc tests revealed that GS39783 at 3 mg/kg significantly increased the distance ratio ($p < 0.01$) and that the dose of 30 mg/kg tended to raise this parameter ($p = 0.066$). Chlordiazepoxide (10 mg/kg) also significantly increased the distance ratio ($p < 0.01$).

Number of stretched attend postures. ANOVA indicated an intergroup difference for the number of stretched attend postures [$F(3,36) = 2.91$; $p < 0.05$]. Post hoc comparisons revealed that GS39783 significantly reduced the number of stretched attend postures at the doses of 3 and 30 mg/kg ($p < 0.05$). Chlordiazepoxide (10 mg/kg) also induced a significant decrease in the number of stretched attend postures ($p < 0.001$).

Number of head dips. The ANOVA indicated that GS39783 significantly modified the number of head dips [$F(3,36) = 5.945$, $p < 0.01$]. Post hoc tests revealed that GS39783 at 3 and 30 mg/kg significantly increased the number of head dips ($p = 0.001$ and $p < 0.01$, respectively). Chlordiazepoxide (10 mg/kg) also sig-

nificantly increased this parameter ($p < 0.001$) (see Fig. 6C).

Effects of GS39783 in the Elevated Plus Maze Test of Anxiety

Ratio. ANOVA indicated a highly significant effect for GS39783 on ratio ($F = 5.849$, $p < 0.001$): all tested doses of GS39783, i.e., 0.1, 1, 10, or 100, induced a significant increase in ratio as compared with vehicle (Fig. 7A). Chlordiazepoxide (10 mg/kg), i.e., the positive standard, also significantly increased the ratio ($p < 0.001$; Fig. 7A).

Total Arm Entries. The ANOVA indicated no significant effect for GS39783 on the total number of arm entries. Chlordiazepoxide (10 mg/kg) significantly increased the number of open arm entries ($p < 0.01$; Fig. 7B).

Time on Open Arms. The ANOVA indicated a highly significant effect for GS39783 on the time spent on open arms ($F = 5.868$, $p < 0.001$): All tested doses of GS39783, i.e., 0.1, 1, 10, or 100 induced a significant increase in time on open arms as compared with vehicle (Fig. 7C). Chlordiazepoxide (10 mg/kg) also significantly increased the time spent on open arms ($p < 0.001$; Fig. 7C).
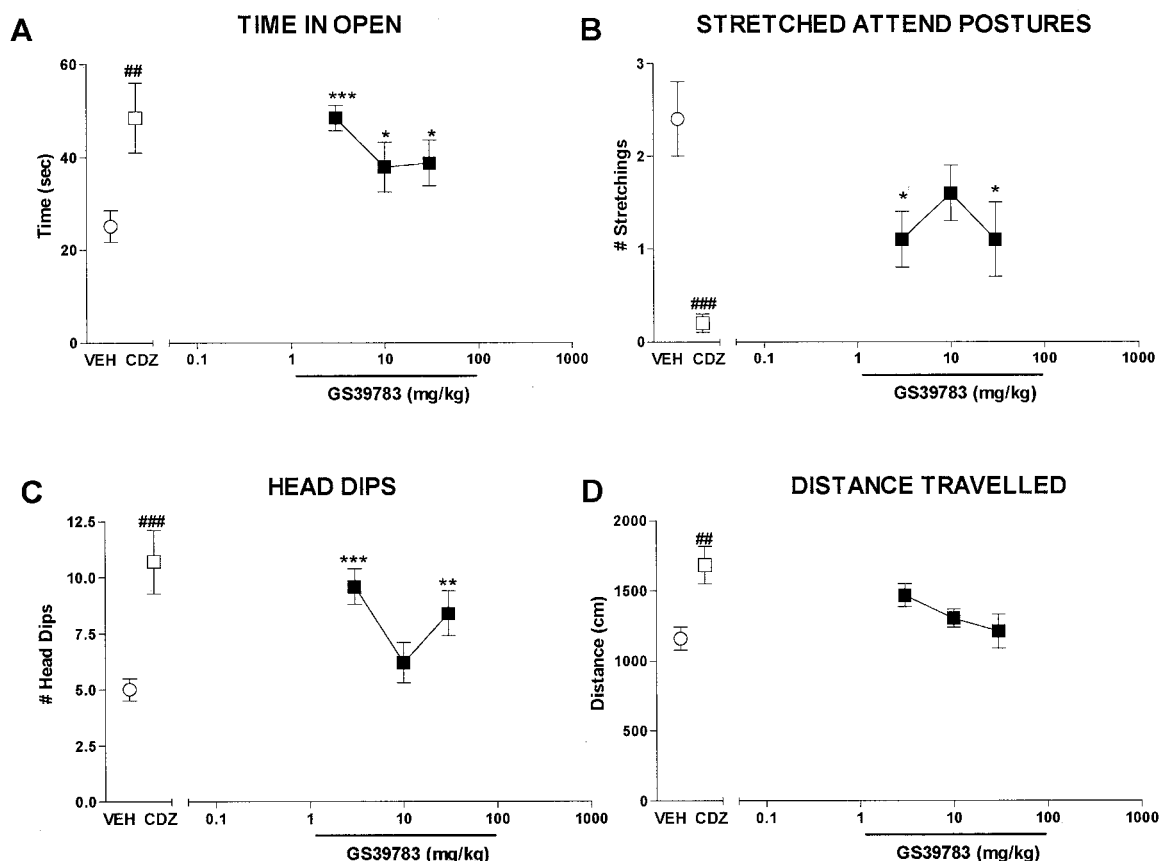
Fig. 6. The effects of GS39783 and chlordiazepoxide on behavior in the rat elevated zero maze. A, time open. Values represent mean (±S.E.M.) of time (seconds) spent in open quadrants during the 5-min test. Drugs were administered p.o. 60 min before the test ($n = 9$–10 rats per group). *, $p < 0.05$; ***, $p < 0.001$ versus vehicle (VEH); Fisher's post hoc tests after ANOVA. ##, $p < 0.01$ versus vehicle; Student's t test. B, stretch-attend postures. Values represent mean (±S.E.M.) of number of stretched attend postures made by animals during the 5-min test. Drugs were administered p.o. 60 min prior to the test ($n = 9$–10 rats per group). *, $p < 0.05$ versus vehicle (VEH); Fisher's post hoc tests after ANOVA. ###, $p < 0.001$ versus vehicle (VEH); Student's t test. C, head dips. Values represent mean (±S.E.M.) of number of head dips made by animals during the 5-min test. Drugs were administered p.o. 60 min prior to the test. **, $p < 0.01$; ***, $p < 0.001$ versus vehicle (VEH); Fisher's post hoc tests after ANOVA. ###, $p < 0.001$ versus vehicle; Student's t test. D, total distance traveled. Values represent mean (±S.E.M.) of total distance (centimeters) traveled during the 5-min test. Drugs were administered p.o. 60 min before the test ($n = 9$–10 rats per group). **, $p < 0.01$ versus vehicle; Fisher's post hoc tests after ANOVA. ##, $p < 0.01$ versus vehicle (VEH); Student's t test.
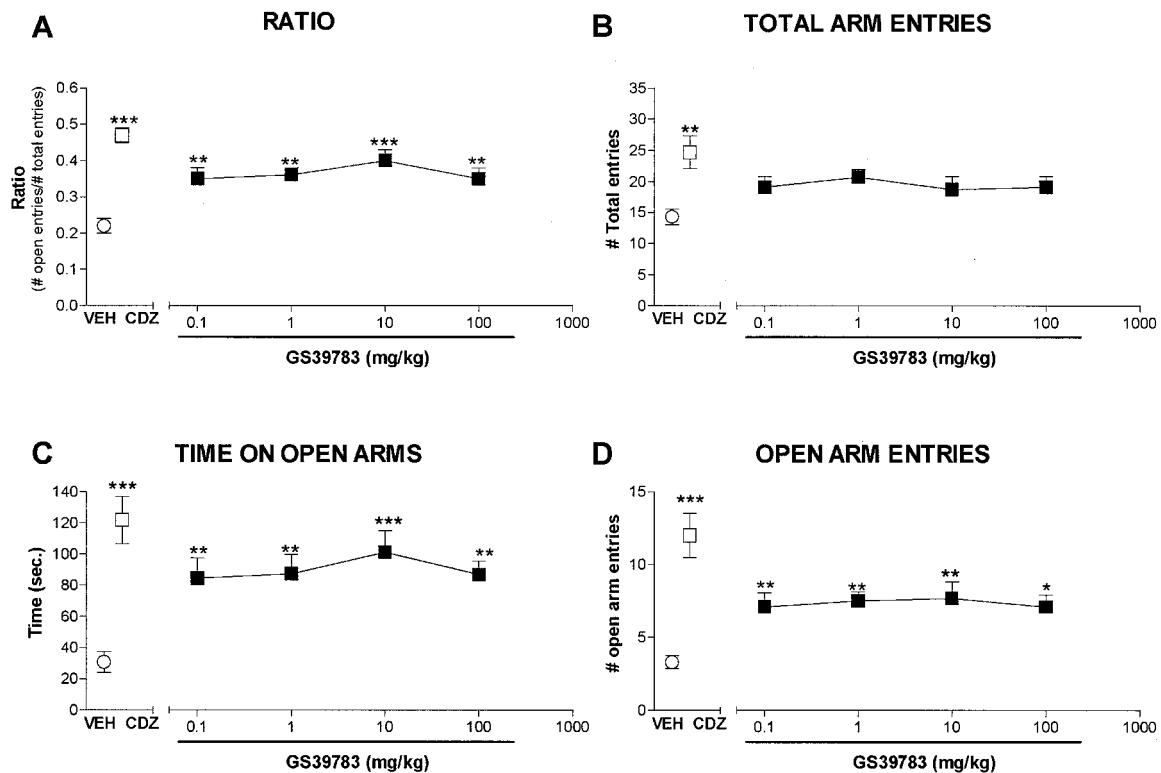
Fig. 7. The effects of GS39783 and chlordiazepoxide on behavior in the rat elevated plus maze. Both the effects of acute GABA$_B$-positive modulator GS39783 (0.1, 1, 10, and 10 mg/kg p.o.) and CDZ (10 mg/kg p.o.) affected the ratio (A), the time spent in open arms (C), and the number of open arm entries compared with vehicle (VEH)-treated animals (D). In contrast, only chlordiazepoxide (10 mg/kg p.o.) increased the total number of arm entries (B) compared with vehicle (VEH)-treated animals. $n = 15$ per treatment group. All values represent mean values, with vertical lines indicating one S.E.M. *, **, and ***, groups that differed significantly from vehicle-treated rats ($p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively).

Open Arm Entries. The ANOVA indicated a highly significant effect for GS39783 on the number of open arm entries ($F = 4.708$, $p < 0.01$). All tested doses of GS39783, i.e., 0.1, 1, 10, or 100, induced a significant increase in number of open arm entries as compared with vehicle (Fig. 7D). Chlordiazepoxide (10 mg/kg) also significantly increased the number of open arm entries ($p < 0.001$; Fig. 7D).

Distance Traveled on Open Arms. The ANOVA indicated a highly significant effect for GS39783 on the distance traveled on open arms ($F = 5.185$, $p < 0.001$): All tested doses of GS39783, i.e., 0.1, 1, 10, or 100, induced a significant increase in distance traveled on open arms as compared with vehicle. Chlordiazepoxide (10 mg/kg) also significantly increased the distance traveled on open arms ($p < 0.001$; data not shown).

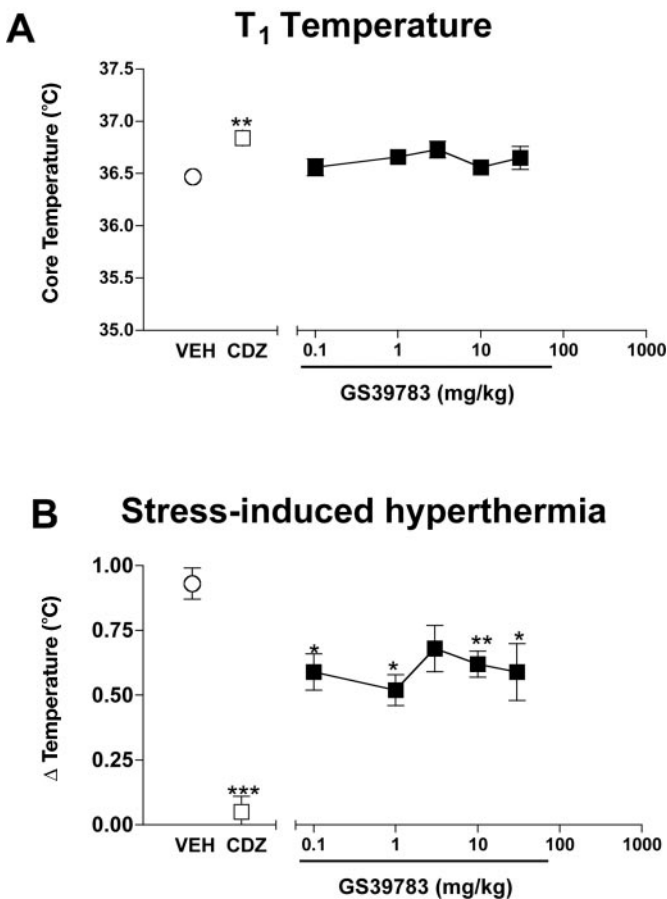### The Effects of GS39783 on Stress-Induced Hyperthermia Test of Anxiety

ANOVA revealed a significant effect of treatment on the magnitude of the SIH response [$F(7,136) = 14.068$, $p < 0.001$]. Post hoc analysis revealed that the positive control chlordiazepoxide (10 mg/kg p.o.) and GS39783 (0.1, 1, 10, and 30 mg/kg) significantly reversed the SIH response (Fig. 8B). Analysis of basal temperature revealed a significant effect of treatment on core body temperature [$F(7,136) = 3.11$, $p < 0.01$]. Post hoc analysis revealed that chlordiazepoxide induced a slight but significant increase in body temperature. In confirmation of experiment 2, GS39783 at any dose tested did not alter baseline body temperature (Fig. 8A).

The Effects of GS39783 on Ethanol-Induced Sedation and Narcosis and on Pentobarbital-Induced Sleep

Rotarod Studies. All of the animals were successfully trained on the rotarod and completed the test prior to injection. Repeated measures ANOVA detected a significant effect of drug treatment [$F(5,108) = 34.407$, $p < 0.001$], a significant effect of ethanol administration [$F(1,108) = 37.422$, $p < 0.001$], a significant drug treatment × ethanol administration [$F(5,108) = 29.081$, $p < 0.001$], a significant effect of time [$F(2,216) = 33.520$, $p < 0.001$], and a significant time × drug treatment × ethanol administration [$F(10,216) = 9.165$, $p < 0.001$]. Post hoc analysis revealed that only animals treated with chlordiazepoxide and ethanol in combination had a marked reduction in endurance on the rotarod. This was maximal 1 h following compound administration but was persistent at 2 h post injection. There was no significant effect 4 h following administration. These data demonstrate that there is no deleterious interaction between GS39783 and ethanol (see Fig. 9).

Narcosis Studies. Irrespective of whether using pentobarbital or ethanol as sleep inducer, GS39783 did not cause any significant change in the sleep duration up to the 100 mg/kg p.o. dose tested. The lack of effect in the second experiment with pentobarbital also ruled out that the trend (not significant) seen following 10 mg/kg GS39783 in the first study was meaningful. In contrast, chlordiazepoxide in all three experiments significantly prolonged the time spent asleep ($p < 0.05$ (Dunn's test versus vehicle group, following significant ANOVA on ranks in all three studies). These data

A

T$_1$ Temperature

B

Stress-induced hyperthermia

Fig. 8. The effects of GS39783, and chlordiazepoxide on stress-induced hyperthermia in mice. A, the effects of GS39783, CDZ, or vehicle (VEH; methylcellulose) on basal temperature 60 min following injection. Values represent mean and S.E.M. (n = 12–24 animals). ∗∗∗, $p \leq 0.001$ versus control group (Vehicle) (Dunnett's post hoc test after ANOVA). B, SIH was assessed as the difference in body temperature 15 min after stress (the taking of rectal temperature is the stressor) compared with baseline temperature, which was determined 60 min after subjects were treated (p.o.) with GS39783, CDZ, or vehicle (VEH; methylcellulose). Values represent mean and S.E.M. (n = 12–24 animals). ∗, $p \leq 0.05$; ∗∗, $p \leq 0.01$; ∗∗∗, $p \leq 0.001$ versus control group (Vehicle) (Dunnett's post hoc test after ANOVA).

point to superiority of GS39783 over chlordiazepoxide, with regard to its lack of hypnotic potential at anxiolytic doses and/or its potential to interact with alcohol (Table 2).

Discussion

In these studies, we sought to behaviorally characterize the prototypical GABA_B receptor modulator GS39783 in paradigms for assessing motor activity, body temperature, cognition, and anxiety. Our data demonstrate that GS39783 is devoid of many of the effects associated with either full GABA_B receptor agonists and/or benzodiazepines; additionally, it displays an anxiolytic profile in a number of rodent models. Taken together, these studies clearly demonstrate that GABA_B receptors play a major role in the modulation of behaviors relevant to anxiety and suggest that positive modulation is a novel approach to probe a role for GABA_B receptors in behavioral processes.

In tests of motor ability (rotarod and locomotor activity), GS39783 is devoid of any sedation compared with baclofen or the anxiolytic agent chlordiazepoxide at doses far above what

is active in other behavioral models. Complimentary data were also obtained using the beam balance test of fine motor behavior and loaded grid test of muscle strength/relaxation (W. P. J. M. Spooren, unpublished data). This demonstrates that use-dependent activation of GABA_B receptors fails to compromise locomotor ability and that GS39783 is not a muscle relaxant. Furthermore, these data strongly suggest that the therapeutic benefits of positive modulators may be greater than that of full agonists for the treatment of disorders where sedation and muscle relaxation may be undesired side effects. However, these data also suggest that GABA_B receptor-positive modulators may not be useful treatments for disorders associated with spasticity, whereas baclofen has been therapeutically successful in this indication for over 30 years (Brogden et al., 1974). GS39783 also differed from baclofen in that it had no intrinsic influence on body temperature, whereas baclofen induces a marked hypothermia. This lack of sedation, muscle relaxation, and hypothermia positions GS39783 as a very useful tool for investigating GABA_B receptor function in various behavioral and physiological paradigms.

The full agonist baclofen and the positive modulator GS39783 also differed on their influence on cognitive function. In the mouse passive avoidance cognition paradigm, baclofen disrupted the performance at doses as low as 1 mg/kg, whereas GS39783 did not have any deleterious effects on cognitive performance in the test. Furthermore, the anxiolytic chlordiazepoxide also negatively affected passive avoidance behavior. In the rat version of the test, GS39783, unlike chlordiazepoxide, was without effect on cognitive performance. Interestingly, no significant deleterious effects of baclofen on cognitive function were seen at the doses tested in the rat passive avoidance test. Reasons for this are presently unclear. Higher doses of baclofen may be required to disrupt performance in the rat test, but we were precluded from using higher doses due to the sedative side effects of baclofen emerging (P. H. Kelly, unpublished data) (also see Fig. 2). Overall, these data are consistent with previous reports demonstrating cognitive impairing effects of both baclofen and chlordiazepoxide on passive avoidance behavior (Swartzwelder et al., 1987; Castellano et al., 1989; Zarrindast et al., 2002). Furthermore, they exemplify the better side effect profile of GABA_B receptor-positive modulators over that of full agonists and benzodiazepine anxiolytics.

Recently, we have shown that mice lacking the GABA_B receptors exhibit an increase in anxiety behaviors (Mombereau et al., 2004), suggesting that activation of GABA_B receptors may decrease anxiety. However, previous data investigating GABA_B mechanisms in anxiety are limited and rather variable; this is largely because investigators relied on using baclofen for such analysis. Baclofen has a narrow efficacy window before confounding side effects are manifested in anxiety paradigms, e.g., in the elevated plus maze it enhanced time spent in the center of the maze, i.e., neither in the open arms or closed arms, which is not interpretable in terms of influencing anxiety (Dalvi and Rodgers, 1996). Nonetheless, baclofen has demonstrated anxiolytic-like effects in a number of tests. It reduced separation-induced calling by mouse pups (Nastiti et al., 1991) and enhanced punished drinking in rats (Ketelaars et al., 1988; Shephard et al., 1992) and had an anxiolytic-like response to novelty in a T maze (Quintero et al., 1985). Furthermore, baclofen also
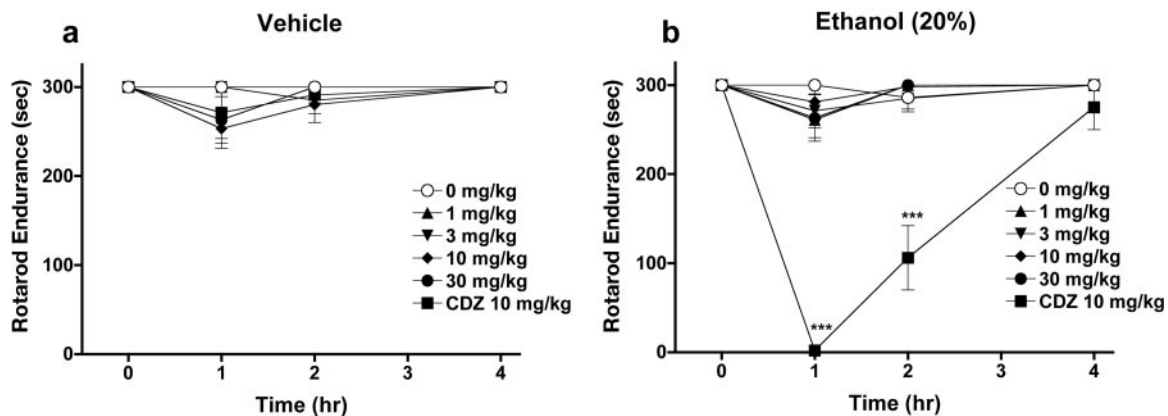
Fig. 9. The effects of GS39783 and chlordiazepoxide on ethanol-induced alterations in rotarod performance in mice. Effects of GS39783 (0, 1, 3, 10, and 30 mg/kg p.o.) and chlordiazepoxide (10 mg/kg p.o.) on rotarod endurance in vehicle-treated mice (a) and ethanol (20%)-treated mice (b). Values represent mean and S.E.M. $n = 10$ per treatment group. ***, groups that differed significantly from vehicle-treated mice ($p < 0.001$).

reversed the anxiogenic response induced by withdrawal from chronic diazepam or alcohol treatment (File et al., 1991, 1992; Andrews and File, 1993). Clinically baclofen reversed the anxiety associated with alcohol withdrawal (Addolorato et al., 2002), posttraumatic stress (Drake et al., 2003), and panic disorder (Breslow et al., 1989).

Because of the above-mentioned data on baclofen, we tested GS39783 in a variety of anxiety models. We first tested the effects of GS39783 in a mouse elevated zero maze because it has been reported that baclofen enhanced time in the center of the more widely used elevated plus maze in mice (Dalvi and Rodgers, 1996). The zero maze has no central area, so the animal must be in either an open or a closed part of the arena, which obviates these potential problems (Shepherd et al., 1994). GS39783 had mild anxiolytic effects in the test (nonsignificant increases in time spent in open quadrants; significant reduction in stretch attend postures), and these effects were not as robust as the anxiolytic chlordiazepoxide. We recently reproduced these mild effects in another strain of mouse, BALB/c (Mombereau et al., 2004). Therefore, we assessed whether these effects of GS39783 were species specific by testing this compound in the rat version of the test. Interestingly, in the rat elevated zero maze, GS39783 had a much more pronounced anxiolytic-like profile (increased time in open quadrants, reduction in stretch-attend postures, and increase in head dips), similar to that of chlordiazepoxide. It is unlikely that a pharmacokinetic explanation underlies the more marked effects in rat versus mouse elevated zero maze because GS39783 is very effective in the mouse SIH test (Fig. 8) and mouse light-dark box test (Mombereau et al., 2004). Nonetheless, these data prompted us to test GS39783 in the more commonly used test of anxiety behavior, the elevated plus maze in the rat. In this test, GS39783 has an anxiolytic profile similar to the benzodiazepine chlordiazepoxide (increased time in open arms, number of entries onto open arms, and the ratio of open/closed arm entries). Furthermore, evidence for anxiolytic-like potential of GS39783 arises from the fact that it attenuates stress-induced hyperthermia in mice, albeit not as markedly as chlordiazepoxide. This paradigm gives a parametric analysis of the physiological response to anxiety (in this case, the anticipatory anxiety caused by an acute stressor) and has been validated extensively as a preclinical paradigm useful to detect conventional and putative anxiolytics (Spooren et al., 2002; Olivier et al., 2003). In addition to the data presented here, we also have recently shown that GS39783 (at doses similar to those employed here) has anxiolytic effects following acute administration in the light-dark box anxiety test (Mombereau et al., 2004) and that these effects persist over 21 days, suggesting that there is no obvious tolerance to its anxiolytic effects.

To be effectively marketed as an anxiolytic, any new chemical entity must show a superior side effect profile to benzodiazepines. In addition to sedation, cognitive effects, and tolerance, one of the primary problems associated with benzodiazepine use is the potentially deleterious interaction with ethanol (Hollister, 1990; Tanaka, 2002). Therefore, we compared the effects of GS39783 with chlordiazepoxide on ethanol-induced changes in two different paradigms: rotarod and sleeping time. To confirm that the effect was not simply

TABLE 2
The effects of GS39783 and chlordiazepoxide (CDZ) on ethanol and pentobarbital-induced sleeping time in mice
Values are means $\pm$ S.E.M. of $n = 14$ to 15 mice per treatment group.

Pretreatment Drug (−60 min)	Experiment A (50 mg/kg i.p. PB)	Experiment B (50 mg/kg i.p. PB)	Experiment C (4 g/kg i.p. EtOH)
		s	
Vehicle	61 ± 3	44 ± 6	18 ± 2
1 mg/kg GS39783	56 ± 3		17 ± 2
3 mg/kg GS39783		58 ± 6	
10 mg/kg GS39783	78 ± 8		21 ± 2
30 mg/kg GS39783		51 ± 6	
100 mg/kg GS39783	59 ± 4		14 ± 2
7.5 mg/kg CDZ	83 ± 7*	91 ± 8*	96 ± 11*

CDZ, chlordiazepoxide; PB, pentobarbital; EtOH, ethanol.
* $p < 0.05$ (Dunn's test vs. vehicle group following significant ANOVA on ranks in all three studies).

pharmacokinetic, we also assessed the effects of both classes of compound on pentobarbital-induced sleep. Chlordiazepoxide at doses that was without effects itself significantly enhanced the ability of ethanol to inhibit motor performance on a rotarod and potentiated both ethanol- and pentobarbital-induced sleeping time. On the other hand, GS39783 was without effect on either of these parameters.

In conclusion, our data suggest that the GABA$_B$ receptor-positive modulator GS39783 has a different behavioral profile compared with that of full agonists, which engender it as a more favorable tool for behavioral or physiological analyses. Furthermore, GABA$_B$ receptor-positive modulators may be a novel avenue for the development of an innovative class of anxiolytic agent devoid of many of the serious side effects associated benzodiazepines. Furthermore, it is tempting to speculate that these positive modulators may be a useful and innovative therapeutic strategy for certain disorders, such as addiction and nicotine dependence, where there is ample evidence that GABA$_B$ receptors play a role, yet baclofen's side effects have thus far limited major therapeutic advances (Brebner et al., 2002; Cryan et al., 2003).

References

Addolorato G, Caputo F, Capristo E, Domenicali M, Bernardi M, Janiri L, Agabio R, Colombo G, Gessa GL, and Gasbarrini G (2002) Baclofen efficacy in reducing alcohol craving and intake: a preliminary double-blind randomized controlled study. *Alcohol Alcohol* **37**:504–508.

Andrews N and File SE (1993) Increased 5-HT release mediates the anxiogenic response during benzodiazepine withdrawal: a review of supporting neurochemical and behavioural evidence. *Psychopharmacology (Berl)* **112**:21–25.

Barnard EA, Skolnick P, Olsen RW, Mohler H, Sieghart W, Biggio G, Braestrup C, Bateson AN, and Langer SZ (1998) International Union of Pharmacology: XV. Subtypes of gamma-aminobutyric acid A receptors: classification on the basis of subunit structure and receptor function. *Pharmacol Rev* **50**:291–313.

Boissier J and Simon P (1960) L'utilisation du test de la traction pour l'étude des psycholeptiques. *Thérapie* **15**:1170–1174.

Bormann J (2000) The "ABC" of GABA receptors. *Trends Pharmacol Sci* **21**:16–19.

Bowery NG, Bettler B, Froestl W, Gallagher JP, Marshall F, Raiteri M, Bonner TI, and Enna SJ (2002) International Union of Pharmacology: XXXIII. Mammalian gamma-aminobutyric acid(B) receptors: structure and function. *Pharmacol Rev* **54**:247–264.

Brebner K, Childress AR, and Roberts DC (2002) A potential role for GABA(B) agonists in the treatment of psychostimulant addiction. *Alcohol Alcohol* **37**:478–484.

Breslow MF, Fankhauser MP, Potter RL, Meredith KE, Misiaszek J, and Hope DG Jr (1989) Role of gamma-aminobutyric acid in antipanic drug efficacy. *Am J Psychiatry* **146**:353–356.

Brogden RN, Speight TM, and Avery GS (1974) Baclofen: a preliminary report of its pharmacological properties and therapeutic efficacy in spasticity. *Drugs* **8**:1–14.

Calver AR, Davies CH, and Pangalos M (2002) GABA(B) receptors: from monogamy to promiscuity. *Neurosignals* **11**:299–314.

Castellano C, Brioni JD, Nagahara AH, and McGaugh JL (1989) Post-training systemic and intra-amygdala administration of the GABA-B agonist baclofen impairs retention. *Behav Neural Biol* **52**:170–179.

Costa E (1989) Allosteric modulatory centers of transmitter amino acid receptors. *Neuropsychopharmacology* **2**:167–174.

Cryan JF, Gasparini F, van Heeke G, and Markou A (2003) Non-nicotinic neuropharmacological strategies for nicotine dependence: beyond bupropion. *Drug Discov Today* **8**:1025–1034.

Dalvi A and Rodgers RJ (1996) GABAergic influences on plus-maze behaviour in mice. *Psychopharmacology (Berl)* **128**:380–397.

Drake RG, Davis LL, Cates ME, Jewell ME, Ambrose SM, and Lowe JS (2003) Baclofen treatment for chronic posttraumatic stress disorder. *Ann Pharmacother* **37**:1177–1181.

Dunham N and Miya TS (1957) A note on a simple apparatus for detecting neuro-logical deficit in rats and mice. *J Am Pharm Assoc Am Pharm Assoc (Baltim)* **46**:208–209.

File SE, Zharkovsky A, and Gulati K (1991) Effects of baclofen and nitrendipine on ethanol withdrawal responses in the rat. *Neuropharmacology* **30**:183–190.

File SE, Zharkovsky A, and Hitchcott PK (1992) Effects of nitrendipine, chlordiazepoxide, flumazenil and baclofen on the increased anxiety resulting from alcohol withdrawal. *Prog Neuropsychopharmacol Biol Psychiatry* **16**:87–93.

Handley SL and Mithani S (1984) Effects of alpha-adrenoceptor agonists and antagonists in a maze-exploration model of 'fear'-motivated behaviour. *Naunyn-Schmiedberg's Arch Pharmacol* **327**:1–5.

Hollister LE (1990) Interactions between alcohol and benzodiazepines. *Rec Dev Alcohol* **8**:233–239.

Jensen AA and Spalding TA (2004) Allosteric modulation of G-protein coupled receptors. *Eur J Pharm Sci* **21**:407–420.

Ketelaars CE, Bollen EL, Rigter H, and Bruinvels J (1988) GABA-B receptor activation and conflict behaviour. *Life Sci* **42**:933–942.

Lecci A, Borsini F, Volterra G, and Meli A (1990) Pharmacological validation of a novel animal model of anticipatory anxiety in mice. *Psychopharmacology (Berl)* **101**:255–261.

Millan MJ (2003) The neurobiology and control of anxious states. *Prog Neurobiol* **70**:83–244.

Mombereau C, Kaupmann K, Froestl W, Sansig G, van der Putten H, and Cryan JF (2004) Genetic and pharmacological evidence of a role for GABA$_B$ receptors in the modulation of anxiety and antidepressant-like behavior. *Neuropsychopharmacology* **29**:1050–1062.

Nastiti K, Benton D, and Brain PF (1991) The effects of compounds acting at the benzodiazepine receptor complex on the ultrasonic calling of mouse pups. *Behav Pharmacol* **2**:121–128.

Olivier B, Zethof T, Pattij T, van Boogaert M, van Oorschot R, Leahy C, Oosting R, Bouwknecht A, Veening J, van der Gugten J, et al. (2003) Stress-induced hyperthermia and anxiety: pharmacological validation. *Eur J Pharmacol* **463**:117–132.

Quintero S, Henney S, Lawson P, Mellanby J, and Gray JA (1985) The effects of compounds related to gamma-aminobutyrate and benzodiazepine receptors on behavioural responses to anxiogenic stimuli in the rat: punished barpressing. *Psychopharmacology (Berl)* **85**:244–251.

Shephard RA, Wedlock P, and Wilson NE (1992) Direct evidence for mediation of an anticonflict effect of baclofen by GABAb receptors. *Pharmacol Biochem Behav* **41**:651–653.

Shepherd JK, Grewal SS, Fletcher A, Bill DJ, and Dourish CT (1994) Behavioural and pharmacological characterisation of the elevated "zero-maze" as an animal model of anxiety. *Psychopharmacology (Berl)* **116**:56–64.

Soudijn W, van Wijngaarden I, and AP IJzerman (2002) Allosteric modulation of G protein-coupled receptors. *Curr Opin Drug Discov Dev* **5**:749–755.

Spooren WP, Schoeffter P, Gasparini F, Kuhn R, and Gentsch C (2002) Pharmacological and endocrinological characterisation of stress-induced hyperthermia in singly housed mice using classical and candidate anxiolytics (LY314582, MPEP and NKP608). *Eur J Pharmacol* **435**:161–170.

Spooren WP, Vassout A, Neijt HC, Kuhn R, Gasparini F, Roux S, Porsolt RD, and Gentsch C (2000) Anxiolytic-like effects of the prototypical metabotropic glutamate receptor 5 antagonist 2-methyl-6-(phenylethynyl)pyridine in rodents. *J Pharmacol Exp Ther* **295**:1267–1275.

Swartzwelder HS, Tilson HA, McLamb RL, and Wilson WA (1987) Baclofen disrupts passive avoidance retention in rats. *Psychopharmacology (Berl)* **92**:398–401.

Tanaka E (2002) Toxicological interactions between alcohol and benzodiazepines. *J Toxicol Clin Toxicol* **40**:69–75.

Tarantino LM, Gould TJ, Druhan JP, and Bucan M (2000) Behavior and mutagenesis screens: the importance of baseline analysis of inbred strains. *Mamm Genome* **11**:555–564.

Urwyler S, Mosbacher J, Lingenhoehl K, Heid J, Hofstetter K, Froestl W, Bettler B, and Kaupmann K (2001) Positive allosteric modulation of native and recombinant gamma-aminobutyric acid(B) receptors by 2,6-Di-tert-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol (CGP7930) and its aldehyde analog CGP13501. *Mol Pharmacol* **60**:963–971.

Urwyler S, Pozza MF, Lingenhoehl K, Mosbacher J, Lampert C, Froestl W, Koller M, and Kaupmann K (2003) N,N'-Dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine (GS39783) and structurally related compounds: novel allosteric enhancers of gamma-aminobutyric acid B receptor function. *J Pharmacol Exp Ther* **307**:322–330.

Van der Heyden JA, Zethof TJ, and Olivier B (1997) Stress-induced hyperthermia in singly housed mice. *Physiol Behav* **62**:463–470.

Venable N and Kelly PH (1990) Effects of NMDA receptor antagonists on passive avoidance learning and retrieval in rats and mice. *Psychopharmacology (Berl)* **100**:215–221.

Zarrindast MR, Bakhsha A, Rostami P, and Shafaghi B (2002) Effects of intrahippocampal injection of GABAergic drugs on memory retention of passive avoidance learning in rats. *J Psychopharmacol* **16**:313–319.

Address correspondence to: Dr. John F. Cryan, Psychiatry Program, Neuroscience Research, The Novartis Institutes for BioMedical Research WSJ 386.344, Novartis Pharma AG, Basel CH-4002, Switzerland. E-mail: john_f.cryan@pharma.novartis.com