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SL651498: An Anxioselective Compound with Functional Selectivity for α_2- and α_3-Containing γ-Aminobutyric Acid$_A$ (GABA$_A$) Receptors

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ABSTRACT

SL651498 [6-fluoro-9-methyl-2-phenyl-4-(pyrrolidin-1-yl-carbonyl)-2,9-dihydro-1*H*-pyrido[3,4-*b*]indol-1-one] is a novel pyridoindole derivative that displays high affinity for rat native GABA$_A$ receptors containing α_1 (K_i = 6.8 nM) and α_2 (K_i = 12.3 nM) subunits, and weaker affinity for α_5-containing GABA$_A$ receptors (K_i = 117 nM). Studies on recombinant rat GABA$_A$ receptors confirm these data (K_i, $\alpha_1\beta_2\gamma_2$ = 17, $\alpha_2\beta_2\gamma_2$ = 73, $\alpha_5\beta_3\gamma_2$ = 215 nM) and indicate intermediate affinity for the $\alpha_3\beta_2\gamma_2$ subtype (K_i = 80 nM). SL651498 behaves as a full agonist at recombinant rat GABA$_A$ receptors containing α_2 and α_3 subunits and as a partial agonist at recombinant GABA$_A$ receptors expressing α_1 and α_5 subunits. SL651498 elicited anxiolytic-like activity similar to that of diazepam [minimal effective dose (MED): 1–10 mg/kg, i.p.] in three conflict models, in the elevated plus-maze, the light/dark test, and the defense test battery in rats and mice. Results from activity tests and electroencephalogram analysis indicated that SL651498 induced muscle weakness, ataxia, or sedation at doses much higher than those producing anxiolytic-like activity (MED $\geq$ 30 mg/kg, i.p.). Repeated treatment for 10 days with SL651498 (30 mg/kg, i.p., b.i.d.) in mice was not associated with the development of tolerance to its anticonvulsant effects or physical dependence. Furthermore, SL651498 was much less active than diazepam in potentiating the depressant effects of ethanol in mice. The "anxioselective" profile of SL651498 points to a major role for GABA$_A$ α_2 subtype in regulating anxiety and suggests that selectively targeting GABA$_A$ receptor subtypes can lead to drugs with increased clinical specificity.

Even though benzodiazepines (BZs) are relatively safe drugs and are widely used in the treatment of anxiety, insomnia, and epilepsy, they may produce untoward side effects such as sedation, muscle relaxation, memory impairment, tolerance, and physical dependence. BZs produce their pharmacological effects through positive allosteric modulation of the action of GABA at ionotropic GABA$_A$ receptors (Braestrup and Squires, 1977; Möhler and Okada, 1977; Barnard et al., 1998). GABA$_A$ receptors have a pentameric structure formed by the assembly of subunits from different families, some of which possess genetic variants (α_{1-6}, β_{1-4}, γ_{1-3}, ρ_{1-3}, ϵ_1, π_1, and δ_1). The existence of at least 16 distinct subunits leads to a substantial GABA$_A$ receptor heterogeneity. The most abundant GABA$_A$ receptor subtypes contain at least one member of the α, β, and γ subunit classes. Sensitivity to BZs is conferred by the γ_2 subunit and adjacent α_1, α_2, α_3, and α_5 subunits.

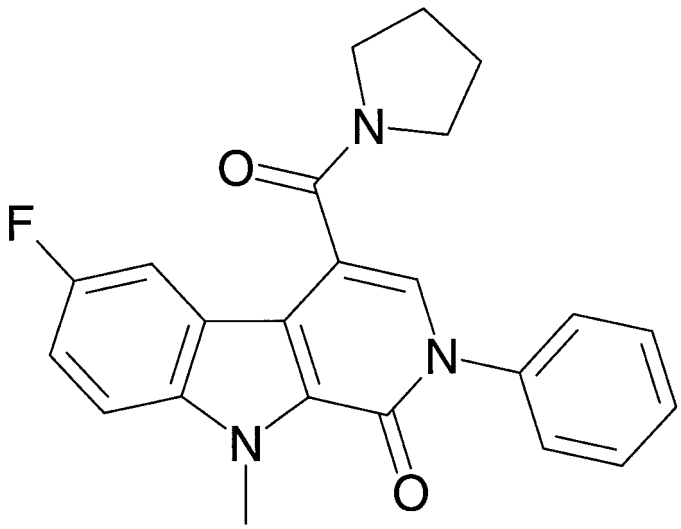
The classical BZs interact indiscriminately with these GABA$_A$ receptor subtypes, hence their myriad of useful and unwanted pharmacological actions. The search for compounds chemically unrelated to the BZs with more specific therapeutic actions and without their concomitant unwanted effects has led to the development of drugs that selectively bind to a specific GABA$_A$ receptor subtype (e.g., the hypnoselective agent, zolpidem, which recognizes preferentially the α_1-containing GABA$_A$ receptor) (Depoortere et al., 1986) or that combine preferential affinity and differential intrinsic activity at these receptors (e.g., abecarnil) (Pribilla et al., 1993). In addition, there are anxioselective compounds that display low efficacies at each GABA$_A$ receptor subtype (e.g., bretazenil, imidazenil, Y-23684) (Martin et al., 1988; Giusti et al., 1993; Yasumatsu et al., 1994). For example, studies in animals showed that the nonselective GABA$_A$ receptor partial agonists bretazenil, imidazenil, and Y-23684 displayed comparable or even greater efficacy in anxiety models than BZs but produced less motor impairment (Martin et al., 1988; Giusti et al., 1993; Griebel et al., 1999b).

The heterogeneity of GABA$_A$ receptors has prompted spec-

ABBREVIATIONS: BZ, benzodiazepine; EEG, electroencephalogram; ANOVA, analysis of variance; DRG, dorsal root ganglion; GABA, γ-aminobutyric acid; HEK, human embryonic kidney; P_{max}, maximal potentiation; VI, variable interval; ECoG, electrocorticogram; SM, sensorimotor; Vis, visual; SL651498, 6-fluoro-9-methyl-2-phenyl-4-(pyrrolidin-1-yl-carbonyl)-2,9-dihydro-1*H*-pyrido[3,4-*b*]indol-1-one; KW, Kruskal-Wallis.

ulation that a particular behavioral response might be associated with an action at a defined receptor subtype. In line with this idea are several investigations that demonstrated that the preferential $GABA_A$ α_1 subtype full agonists zolpidem and abecarnil induce myorelaxant effects at doses that are much higher than those decreasing exploration or inducing sleep, while nonselective $GABA_A$ receptor full agonists primarily affect muscle strength, suggesting that myorelaxant activities are not related to an interaction with α_1-containing $GABA_A$ receptors (Perrault et al., 1990; Griebel et al., 1999b). Moreover, preferential $GABA_A$ α_1 subtype full agonists are generally found to display weaker (if any) anxiolytic-like activity in animals than nonselective agents (e.g., Sanger, 1995; Griebel et al., 1999b). Studies using mice with point-mutated zolpidem- or diazepam-insensitive $GABA_A$ receptor subtypes showed that the sedative action of zolpidem was absent in α_1 point-mutation mice (Crestani et al., 2000; Low et al., 2000), whereas the anxiolytic-like action of the nonselective $GABA_A$ receptor agonist diazepam was absent in α_2 but not in α_1 or α_3 mice (Rudolph et al., 1999). Together, these findings indicated that the sedative and anxiolytic effects of $GABA_A$ receptor agonists are mediated by the α_1 and α_2 $GABA_A$ receptors, respectively.

In the present article, we report on the preclinical pharmacological profile of SL651498 [6-fluoro-9-methyl-2-phenyl-4-(pyrrolidin-1-yl-carbonyl)-2,9-dihydro-1*H*-pyrido[3,4-*b*]indol-1-one] (Fig. 1), a pyridoindole derivative selected on the basis of its functionally selective agonist activity at the α_2 and α_3 $GABA_A$ receptor subtypes. Some biochemical and electrophysiological properties of the drug are presented together with its profile in a variety of behavioral tests, including models of anxiety, anticonvulsant activity, motor activity, drug discrimination, physical dependence, and ethanol interaction in rodents. Comparative data for the prototypical BZ diazepam, obtained under the same experimental conditions, are also provided.



SL651498

Fig. 1. Chemical structure of SL651498 [6-fluoro-9-methyl-2-phenyl-4-(pyrrolidin-1-yl-carbonyl)-2,9-dihydro-1*H*-pyrido[3,4-*b*]indol-1-one].

Materials and Methods

Animals

Male Sprague-Dawley or Wistar rats (Iffa Credo, L'Arbresle and Charles River, Saint-Aubin-lès-Elbeuf, France) weighing 100 to 600 g at the time of testing were used in the punished lever pressing and drinking procedures, the elevated plus-maze test, the drug discrimination study, the rotarod, the grip strength test, and for EEG and in vitro radioligand binding studies. Rats used in the punished drinking procedure, the elevated plus-maze test, the rotarod, and the grip strength test and those used in radioligand binding studies were housed in groups of eight, whereas those used in the punished lever pressing and drug discrimination procedures were housed singly. In electrophysiological experiments, dorsal root ganglion cells were prepared from Sprague-Dawley rats (1-day-old, Charles River France). Male CD1 (isoniazid-induced convulsions, rotarod, and grip strength test), OF1 (pentylenetetrazole-induced convulsions and mouse defense test battery), NMRI (four-plate test), and BALB/c (light/dark test) mice weighing 18 to 32 g were supplied by Charles River, Iffa Credo, or Janvier (Le Genest, France). CD1, OF1, and NMRI mice were housed in groups of 20, those used in the mouse defense test battery were housed singly, and BALB/c mice were housed in groups of 6. All animals were maintained under standard laboratory conditions (21–22°C, 40–60% relative humidity) with free access to food and water. They were kept on a 12:12-h light/dark cycle with light onset at 6:00 AM.

Biochemical and Electrophysiological Studies

In Vitro Binding of [^{3}H]Flumazenil to Different BZ-Sensitive $GABA_A$ Receptors. Inhibition of [^{3}H]flumazenil binding to rat native $GABA_A$ receptor subtypes in vitro was performed as described in Schoemaker et al. (1997). Briefly, the cerebellum, spinal cord, or hippocampus was homogenized in 50 mM Tris-HCl buffer (pH 7.4) containing 120 mM NaCl and 5 mM KCl. The binding of [^{3}H]flumazenil (1 nM; specific activity, 70–87 Ci/mmol) to the α_1-containing $GABA_A$ receptor was studied in membranes from the cerebellum, a region enriched in this receptor subtype (Braestrup and Nielsen, 1980), using a 45-min incubation at 0–4°C and 1 μM diazepam to define nonspecific binding. [^{3}H]Flumazenil binding to the $GABA_A$ receptor expressing the α_2 subunit was studied using membranes from the rat spinal cord, where a majority of the expressed $GABA_A$ receptors appear to be of the α_2 subtype (Ruano et al., 1992), under otherwise identical conditions. The native α_5-containing $GABA_A$ receptor was studied using [^{3}H]flumazenil binding to membranes from the rat hippocampus in the presence of 5 μM zolpidem to mask the α_1 and α_2/α_3 subtypes (Tan and Schoemaker, 1994), under otherwise identical conditions except for the use of 1 μM flunitrazepam to define nonspecific binding. Following incubation, membranes were recovered by vacuum filtration over Whatman (Maidstone, UK) GF/B filters and washed, and the amount of radioactivity retained on the filter was quantified by liquid scintillation spectrometry. [^{3}H]Flumazenil binding to recombinant rat $\alpha_1\beta_2\gamma_2$-, $\alpha_2\beta_2\gamma_2$-, $\alpha_3\beta_2\gamma_2$-, and $\alpha_5\beta_3\gamma_2$-containing $GABA_A$ receptor was studied in stably transfected HEK293 cells essentially as described by Besnard et al. (1997). Nonspecific binding was defined using 10 μM diazepam. The concentrations of SL651498 and diazepam tested ranged from 10^{-10} to 10^{-6} M.

Electrophysiological Studies. To compare the functional properties of SL651498, diazepam, and zolpidem at $GABA_A$ receptors, we performed whole-cell patch-clamp experiments with HEK293 cells stably expressing recombinant rat $\alpha_1\beta_2\gamma_2$, $\alpha_2\beta_2\gamma_2$, $\alpha_3\beta_2\gamma_2$, or $\alpha_5\beta_3\gamma_2$ subunits, and with rat dorsal root ganglion (DRG) neurons in culture, which contain exclusively native α_2-containing $GABA_A$ receptors (Ma et al., 1993).

Cell preparations: Cell lines expressing $\alpha_1\beta_2\gamma_2$, $\alpha_2\beta_2\gamma_2$, $\alpha_3\beta_2\gamma_2$, or $\alpha_5\beta_3\gamma_2$ $GABA_A$ receptor combinations were maintained in appropriate culture conditions (Besnard et al., 1997). Cells were scraped from the culture flask and transferred to a plastic-bottomed patch-clamp recording chamber in which they settled. The chambers were placed on the stage of an inverted microscope (Olympus IMT2) equipped with Hoffman optics (Modulation Contrast, New York, NY) and the cells viewed at a total magnification of 400×. A polyethylene tube (500-μm opening) connected to a solution distributor was moved to within 3 mm of the cell under investigation and allowed fast superfusion of solutions (3–5 ml/min). Primary cultures of neonatal rat DRG cells were prepared from 1-day-old pups. Briefly, after dissection, the DRG were trypsinized and the cells were dissociated by gentle trituration. Cells were resuspended in basal Eagle's culture medium containing 10% fetal bovine serum, 25 mM KCl, 2 mM glutamine, 100 μg/ml gentamicin, and 50 ng/ml of nerve growth factor, then seeded on laminin-coated glass coverslips (0.25×10^6 cells/coverslip), and placed in 12-well Corning dishes. Cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2/95% air. Cytosine β-D-arabinoside (1 μM) was added 48 h after seeding to prevent the replication of non-neuronal cells. The coverslips were transferred to the experimental chambers for patch-clamp experiments after 7 to 10 days in culture.

Patch-clamp: The whole-cell configuration of the patch-clamp technique was used. Pipettes were pulled from thick-walled borosilicate glass capillaries (Phymep, Paris, France) on a two-stage puller and had a resistance of 5 to 10 MΩ when filled with the pipette solution. Pipettes were brought into contact with the cells with a three-dimensional piezoelectric micromanipulator (Burleigh PCS1000, Optilas, Evry, France). Whole-cell currents were recorded with an Axopatch 1D (Axon Instruments, Burlingame, CA) connected to a 386 DX personal computer (Compaq) driven by pClamp software (Axon Instruments). Chloride currents were elicited by concentrations of GABA giving 5 to 10% of the maximal response, i.e., 0.3 μM for $\alpha_1\beta_2\gamma_2$-transfected cells, 1 μM for $\alpha_3\beta_2\gamma_2$- and $\alpha_5\beta_3\gamma_2$-transfected cells, and 3 μM for $\alpha_2\beta_2\gamma_2$-transfected cells. For each cell, the potentiations were measured by comparing the chloride current obtained in response to GABA alone with that obtained in the presence of SL651498, zolpidem, or diazepam.

Absolute potentiation factors are the ratio between the amplitude of the GABA-induced current in the presence or in the absence of SL651498. Relative potentiation factors are the ratio between the potentiation in the presence of SL651498, and the maximal potentiation (P_{max}) induced by the reference compound. The P_{max} value reflects the efficacy of the compounds. Means are given with standard error of the mean (S.E.M.). For concentration-response curves, the nonlinear curve-fitting routine (by Levenberg-Marquard iterations) of the Origin software (MicroCal Software Inc., Northampton, MA) was used, and the Hill coefficient value was fixed at 1. Parameters providing the best fit are given ± S.E.M.

Anticonvulsant Activity of SL651498

Antagonism of Pentylenetetrazole-Induced Convulsions in Mice. At 30 min after intraperitoneal (i.p.) injection of the test drugs or the vehicle, mice were given a subcutaneous (s.c.) injection of 125 mg/kg pentylenetetrazole. The occurrence of tonic extension of the hindlimbs was noted during the 30-min period that followed. ED_{50} values were calculated using the probit method of Litchfield and Wilcoxon (1949).

Antagonism of Maximal Electroshock-Induced Convulsions in Mice. Following i.p. injection of SL651498 or diazepam, electroshock seizures were produced by an electric current (60 mA, 50 Hz, 0.4 s) delivered through a pair of corneal electrodes. The occurrence of tonic extension of the hindlimbs was noted. ED_{50} values were calculated using the log-probit method of Litchfield and Wilcoxon (1949).

Antagonism of Isoniazid-Induced Convulsions in Mice. Isoniazid (800 mg/kg, s.c.) was administered simultaneously with SL651498, diazepam, or zolpidem (i.p.). The anticonvulsant effect was assessed by measuring the latency to the appearance of the first convulsion. In a first series of experiments, SL651498 and diazepam were administered alone. In a second set of experiments, SL651498 was coadministered with zolpidem or diazepam. In the interaction studies, SL651498 (30 mg/kg) and zolpidem (3 and 10 mg/kg) or diazepam (3 and 10 mg/kg) were tested alone or in combination. Finally, in a third experiment, isoniazid was administered simultaneously with SL651498, and the BZ receptor antagonist flumazenil was injected 15 min later. Data were analyzed using a one-way ANOVA followed by Dunnett's t test, Newman-Keuls test, or Student's t test. ED_{50} values were calculated by linear regression.

Anxiolytic-Like Activity of SL651498

Punished Lever Pressing Test in Rats. Animals were restricted to the food obtained during sessions and a daily ration of 15 to 20 g of standard laboratory chow given at the end of each weekday and over the weekend. The procedure was a modification of that described previously (Sanger et al., 1985). Animals were tested in standard rat operant test chamber (MED Associates, Inc., St. Albans, VT) placed in sound-attenuated boxes that were well ventilated. Each chamber was fitted with a stainless steel grid floor. Electric shocks could be delivered to each grid by a shock generator and scrambler (MED Associates, Inc.). A total of 11 rats were trained initially to press a lever for food reward (45-mg precision food pellets, PJ Noyes, Inc., Lancaster, NH). As training progressed, schedule parameters were gradually changed to a variable interval (VI 30 s) schedule of food reinforcement during daily 15-min sessions. After several sessions of VI 30 s responding, five 60-s periods of a visual stimulus were presented during a 25-min session. Each visual stimulus consisted of three stimulus lights situated above the food pellet dispenser and to the right of the response lever, which flashed at a rate of 1 s on, 1 s off. In this component, a footshock punishment schedule consisting of two independent VI schedules (VI 30 s for food, VI 10 s for shock) was in operation. Footshock was initially set at 0.1 mA. The first stimulus presentation started 5 min after the beginning of the session, and each following stimulus commenced 150 s after the end of the preceding stimulus. The magnitude of footshock was individually titrated for each rat (shock levels ranged from 0.3–0.65 mA) to obtain stable baselines of responding (i.e., an average lever pressing rate of eight ± two presses in each 1-min punished responding period). To obtain stable levels of responding, an average of approximately 30 sessions after initiation of the punishment contingency was necessary. Once stable baselines of responding were obtained, drug studies were initiated.

Drug injections were given once or twice each week with at least 2 nondrug days intervening between two drug administrations. Vehicle was injected on all nondrug days. Drugs and doses were given in a mixed order. The effects of drugs were assessed on punished and unpunished responses rates. The former correspond to those recorded during the presentation of the visual stimulus, whereas the latter were taken from the 60-s periods immediately preceding and immediately following each stimulus presentation. The mean values of punished and unpunished rates recorded during the nondrug session preceding the drug injection sessions were used as the control values. Thus, drug effects were analyzed statistically by comparing performances after drug administration with the mean values taken from appropriate control sessions using a Friedman's ANOVA. Experiments were performed 30 min after i.p. injection of the drugs. In a second experiment, SL651498 (10 mg/kg, i.p.) and flumazenil (10 mg/kg, i.p.) were tested alone or in combination. Flumazenil was injected 15 min after the administration of SL651498, and experiments started 30 min after injection of SL651498. Effects were analyzed statistically by comparing performances after drug administration with the mean values taken from appropriate control sessions using a Friedman's ANOVA.

Punished Drinking Test in Rats. The procedure was a modification of the technique described by Vogel et al. (1971). At the

beginning of the experiment, rats (180–230 g; 35–41 days old), deprived of water for 48 h prior to testing, were placed in cages (32 × 25 × 30 cm) with a stainless steel grid floor. Each cage was placed in sound-attenuated boxes that were well ventilated and contained a drinking tube connected to an external 50-ml buret filled with tap water. Trials were started only after the animal's tongue entered in contact with the drinking tube for the first time. An electric shock (0.06 mA/10 ms) was delivered to the tongue after every 20 licks. The number of shocks was recorded automatically during a 5-min period. Results were analyzed by the nonparametric Kruskal-Wallis test. Experiments were performed 30 or 60 min after i.p. or p.o. injection of the drugs, respectively.

Elevated Plus-Maze Test in Rats. The test apparatus is based on that described by Pellow et al. (1985). All parts of the apparatus were made of dark polyvinylplastic with a black rubber floor. The maze was elevated to a height of 50 cm with two open (50 × 10 cm) and two enclosed arms (50 × 10 × 50 cm), arranged so that the arms of the same type were opposite each other, connected by an open central area (10 × 10 cm). To prevent rats falling off, a rim of Plexiglas (1 cm high) surrounded the perimeter of the open arms. The illumination in the experimental room consisted of one red neon tube fixed on the ceiling, so that experiments were performed under dim light conditions. The light intensity on the central platform was 10 lux. At the beginning of the experiment, rats were placed in the center of the maze, facing one of the enclosed arms, and observed for 4 min. The apparatus was equipped with infrared beams and sensors capable of measuring time spent in open arms, number of open-arm entries, and number of closed-arm entries (defined as entry of all four limbs into an arm of the maze). In addition, rats were observed via video link by an observer located in an adjacent room. This permitted the recording of a more ethologically orientated measure: attempt at entry into open arms followed by avoidance responses. This includes stretched attend posture (the rat stretches forward and retracts to original position). The results were expressed as mean ratio of time spent in open arms to total time spent in both open and closed arms, mean ratio of entries into open arms to total entries into both open and closed arms, mean total number of both closed and open arm entries, and mean total number of attempts. Data were analyzed with one-way ANOVA. Subsequent comparisons between treatment groups and control were carried out using Dunnett's *t* test. Experiments were performed 30 or 60 min after i.p. or p.o. injection of the drugs, respectively. In a second experiment, the duration of the anxiolytic-like action of 10 mg/kg (p.o.) SL651498 was investigated. Rats were injected with the compound and placed on the elevated plus-maze test at 30 min, 1 h 30, 3 h, or 6 h later. Each rat was tested once. Data were analyzed with one-way ANOVA. Subsequent comparisons between treatment groups and control were carried out using Dunnett's *t* test.

Light/Dark Test in Mice. The test apparatus is based on that described by Misslin et al. (1989). It consisted of two polyvinylchloride boxes (20 × 20 × 14 cm) covered with Plexiglas. One of these boxes was darkened. A neon tube fixed on the ceiling provided the room illumination so that the light intensity in the center of the illuminated box was 200 lux. An opaque plastic tunnel (5 × 7 × 10 cm) separated the dark box from the illuminated one. At the beginning of the experiment, a mouse was placed in the illuminated box, facing the tunnel. Recording started when the animal entered the tunnel for the first time. The apparatus was equipped with infrared beams and sensors capable of recording the following parameters during a 4-min period: (a) time spent by mice in the lit box; (b) attempt at entry into the lit box followed by avoidance responses, including stretched attend posture (the mouse stretches forward and retracts to original position); and (c) total number of tunnel crossings. Data were analyzed with the Kruskal-Wallis test. Experiments were performed 30 min after i.p. injection of the drugs.

Four-Plate Test in Mice. The test apparatus is based on that described by Boissier et al. (1968). The apparatus consisted of a cage with a floor composed of four rectangular metal plates connected to a device that can generate electric shocks (1 mA; 0.2 s). Following a 15-s latency period, the animal was subjected to an electric shock every time it went from one plate to another. The number of punished crossings is recorded during a 1-min test period. Data were analyzed by a one-way ANOVA. Subsequent comparisons between treatment groups and control were carried out using Dunnett's *t* test. Experiments were carried out 30 or 60 min after i.p. or p.o. injection of the drugs, respectively.

Mouse Defense Test Battery. The test was conducted in an oval runway, 0.4 m wide, 0.3 m high, and 4.4 m in total length, consisting of two 2-m straight segments joined by two 0.4-m curved segments and separated by a median wall (2 × 0.3 × 0.06 m). The apparatus was elevated to a height of 0.8 m from the floor to enable the experimenter to easily hold the rat, while minimizing the mouse's visual contact with him. All parts of the apparatus were made of black Plexiglas. Activity was recorded with video cameras mounted above the apparatus.

Procedure: (a) *Pretest: 3-min familiarization period*. Thirty or 60 min after i.p. or p.o. injection of the drugs, respectively, subjects were placed into the runway for a 3-min familiarization period, in which line crossings were recorded. (b) *Chase/flight test*. A hand-held dead rat (killed by CO_2 inhalation) was brought up to the subject at a speed of approximately 2 m/s. A constant distance of 2 m separated the rat and the subject when the former was introduced in the runway. Chase was initiated only when the subject was at a standstill with its head oriented toward the hand-held rat. Chase was completed when the subject had traveled a distance of 15 m. During the chase, a constant distance of 20 cm was maintained between the two animals. Consequently, if the animal stopped fleeing before traveling the full 15 m, the chase was stopped too in order to avoid contact between the two animals. The experimenter then moved the hand-held rat quickly from left to right in front of the subject to elicit flight. The following parameters were recorded: number of stops (pause in movement) and orientations (subject stops, then orients the head toward the rat). The rat was removed after the chase was completed. (c) *Straight alley*. By the closing of two doors (60 cm distant from each other), the runway was then converted to a straight alley in which the subject was constrained. The rat was introduced in one end of the straight alley. Session was initiated when 1) the subject faced the rat; and 2) both animals were 40 cm distant from each other. During 30 s, the number of approaches/withdrawals (subject must move more than 20 cm forward from the closed door, then return to it) was recorded. The hand-held rat remained at the place it was introduced during the full 30 s. After this session, it was removed from the straight alley area. (d) *Forced contact*. Finally, the experimenter brought the rat up to contact the subject in the straight alley. Approaches were directed quickly (within 1 s) to the subject's head. For each such contact, bites by the subjects were noted. If no defensive threat and/or attack responses were elicited within 15 s, the rat was removed from the apparatus. This was repeated three times. The time interval between each trial was approximately 5 ± 1 s. (e) *Post-test: contextual defense*. Immediately after the forced contact test, the rat was removed and the doors opened. Escape attempts were recorded during a 3-min session. Data were analyzed by a one-way ANOVA. Subsequent comparisons between treatment groups and control were carried out using Dunnett's *t* test.

Discriminative Stimulus Properties in Rats

Subjects were trained to discriminate a dose of 5 mg/kg i.p. chlordiazepoxide or a dose of 2 mg/kg i.p. zolpidem from saline using a standard, two-lever, fixed ratio 10, food-rewarded operant procedure. Thus, rats obtained a food pellet (45 mg) each time they pressed 10 times on the appropriate lever in the two-lever operant test chamber. Responses on one lever were rewarded in sessions that followed chlordiazepoxide or zolpidem injection, and responses on the other lever were rewarded during the session following saline injection (see Sanger and Zivkovic, 1986, for further details of the procedure).

Daily sessions were 15 min in duration. When the animals had acquired the generalization, they were given substitution tests with a range of doses of chlordiazepoxide or zolpidem. The rats were then tested with several doses of SL651498 or diazepam. The drugs were administered i.p. 30 min before the beginning of the test, except zolpidem, which was given 15 min before the session. The results were recorded as the number of rats choosing the drug-associated lever during the substitution tests. Data were analyzed using the probit method of Litchfield and Wilcoxon (1949) to calculate the ED_{50} values. The ED_{50} discrimination is the dose at which 50% of the rats responded on the drug-associated.

Side-Effect Profile of SL651498

Rotarod Test in Mice and Rats. Mice were pretested on the rotarod (turning at 10 turns/min, diameter of 3 cm), and animals that stayed on the apparatus for 2 min were selected for drug testing. Each animal was given a maximum of two trials during the pretest. Approximately 2 h later, each animal was injected i.p. with SL651498 or diazepam and was placed 30 min later on the rotarod. The time each mouse stayed on the rotarod was recorded up to a maximum of 2 min. Rats were trained to stay on a rotarod turning at a speed of 5 turns/min (diameter of 6 cm) for at least 1 min. It was not necessary to eliminate any animals. Approximately 3 h later, a second pretest was given during which the apparatus first turned at the same constant speed for 1 min and then turned at an accelerating speed (5–42 turns/min in 10 min). Twenty-four hours later, each rat was injected i.p. with SL651498 or diazepam and, 30 min later, was placed again on the rotarod. The length of time each animal stayed on the apparatus at accelerating speed was recorded. Data were analyzed by a one-way ANOVA. Subsequent comparisons between treatment groups and control were carried out using Dunnett's *t* test. ED_{50} values were calculated by linear regression.

Grip Strength Test in Mice and Rats. This test is based on that described previously by Meyer et al. (1979). The grip strength apparatus is constructed from Plexiglas. The base is 66 cm long and 23 cm wide and has a 29- × 23-cm platform mounted 15 cm above the base on a plus-shaped pedestal. Two adjustable 7.6-cm high L-shaped guides are mounted across the entire length of the platform to form an adjustable trough. Commercially available push-pull strain gauges (Phymep, Châtillon, France) are positioned at the end of each platform and secured horizontally via pretapped holes in the body of the gauge to vertically adjustable pedestals mounted on the Plexiglas base. The strain gauge used to measure forelimb grip strength has a 7.6-cm equilateral triangular brass ring soldered onto a hexagonal aluminum standoff, which threads onto an extension arm supplied with the strain gauge. Thirty minutes after an i.p. injection, the animal is placed into the trough with the forepaws inside the triangular grasping ring. Using one hand, the animal is grasped about 3/4 of the way up toward the base of the tail and steadily pulled (≈2 cm/s) away from the ring until the grip is broken. Typically, five successive readings are taken for each animal with an intertrial interval of 10 s. Results are expressed as mean grip strength (g) − weight of the animal (mice: 30 g; rats: 200 g). ED_{50} values were calculated by linear regression.

Electrocorticogram (ECoG) Studies. Rats were anesthetized with halothane 4% in a Plexiglas box, immobilized by alcuronium (5 mg/kg, i.p.), and then artificially ventilated with air using a mask over the muzzle. They were mounted in a stereotaxic apparatus. During the implantation of cortical electrodes, anesthesia was maintained under halothane (0.5%), and local infiltrations with lidocaine 2% were performed at all pressure and incision points. Body temperature was maintained at 37.5°C. The ECoG was recorded using small stainless steel screw electrodes (0.9 mm in diameter). Cortical electrodes were screwed into the bone over the sensorimotor cortex (1.5 mm lateral to the median suture and 1.5 mm behind the frontoparietal suture), the visual cortex (1.5 mm lateral to the median suture and 1.5 mm in front of the parieto-occipital suture), and over the cerebellum (reference electrode). The activity in the sensorimotor (SM) and visual (Vis) cortices were recorded by comparison with the reference electrode placed over the cerebellar cortex. At the end of the surgical preparation, the administration of halothane was discontinued and ECoGs were recorded after a recovery period of 60 min. The EEG was amplified and filtered (1–16 Hz, 48 dB/oct). After a 30-min control period, increasing doses of drug were administered by i.p. or p.o. route at 30-min intervals. The sequential spectral analysis of 30-s periods was performed on SM and Vis recordings using a Berg-Fourier analyzer (1–16 Hz, 48 dB/oct). In rats, six frequency bands were defined: delta (1–4 Hz), theta (4.5–7 Hz), alpha 1 (7.5–9.5 Hz), alpha 2 (10–12.5 Hz), beta 1 (13–18 Hz), and beta 2 (18.5–32 Hz). The treatment of the rat with alcuronium produced a stable ECoG recording of wakefulness, characterized by a low cortical theta activity (5–6 Hz).

For each rat, the quantitative ECoG analysis was performed on the SM lead. Several parameters were calculated for every 5-min period: 1) mean total power (TP) for the band 1 to 32 Hz in μV^2; 2) mean absolute power for each frequency band (BP); and 3) the absolute power in percent for each frequency band (AP%) and for the total power (TP%): $AP\% = BP/TP_0 \times 100$, $TP\% = TP/TP_0 \times 100$, TP_0 is the total power of the control period.

Sleep-Wakefulness Cycle Studies in Chronically Implanted Rats. Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and mounted in a stereotaxic apparatus. The implantation of electrodes was similar to that described above. Cortical electrodes were attached to a connector (Winchester, 7-lead) and fixed with dental cement to the cranium. After 3 weeks of postoperative recovery, animals were placed in Plexiglas cylinders (60 cm in diameter) with free access to food and water. The temperature of the room was kept constant (21 ± 1°C), and darkness was from 7:00 AM to 7:00 PM. The rats were recorded from 11:00 AM to 5:00 PM during 3 consecutive days (control day, drug day, and control day). Activity in SM and Vis cortices was recorded by comparison with a reference electrode placed in the cerebellar cortex. Three stages were differen-

TABLE 1
Effects of SL651498 on the binding of [^{3}H]flumazenil to recombinant rat $GABA_A$ receptors, and to native rat $GABA_A$ receptors in the cerebellum, the spinal cord, and the hippocampus, which predominantly express α_1, α_2, and α_5 subtypes, respectively

	$GABA_A$ Receptor Subtype			
	K_i			
	nM			
Native[a]	α_1	α_2		α_5
SL651498	6.8 ± 1.9	12.3 ± 1.9		117 ± 13[b]
Diazepam	5.8 ± 1.9	4.8 ± 1.9		13.4 ± 4.7[b]
Recombinant	$\alpha_1\beta_2\gamma_2$	$\alpha_2\beta_2\gamma_2$	$\alpha_3\beta_2\gamma_2$	$\alpha_5\beta_3\gamma_2$
SL651498	17.0 ± 1.5	73.0 ± 7.0	80.3 ± 10.4	215 ± 7
Diazepam	14.0 ± 2.1	7.8 ± 1.1	13.9 ± 0.5	9.8 ± 1.1

[a] Data represent the mean of at least three experiments performed in duplicate.
[b] Studied in the presence of 5 μM zolpidem to mask the $GABA_A$ receptor α_1 and α_2 subtypes.

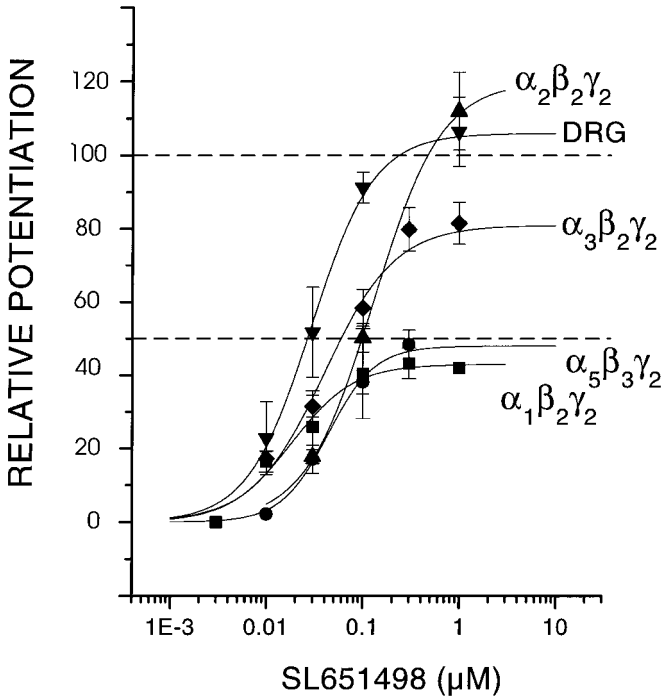


Fig. 2. GABA-induced chloride currents potentiation by SL651498 relative to zolpidem (■, $\alpha_1\beta_2\gamma_2$) or diazepam (▲, $\alpha_2\beta_2\gamma_2$; ♦, $\alpha_3\beta_2\gamma_2$; ●, $\alpha_5\beta_3\gamma_2$) in HEK293 cells stably expressing rat recombinant $GABA_A$ receptor subtypes, and rat DRG neurons in culture which contain exclusively native α_2-containing $GABA_A$ receptors (▼).

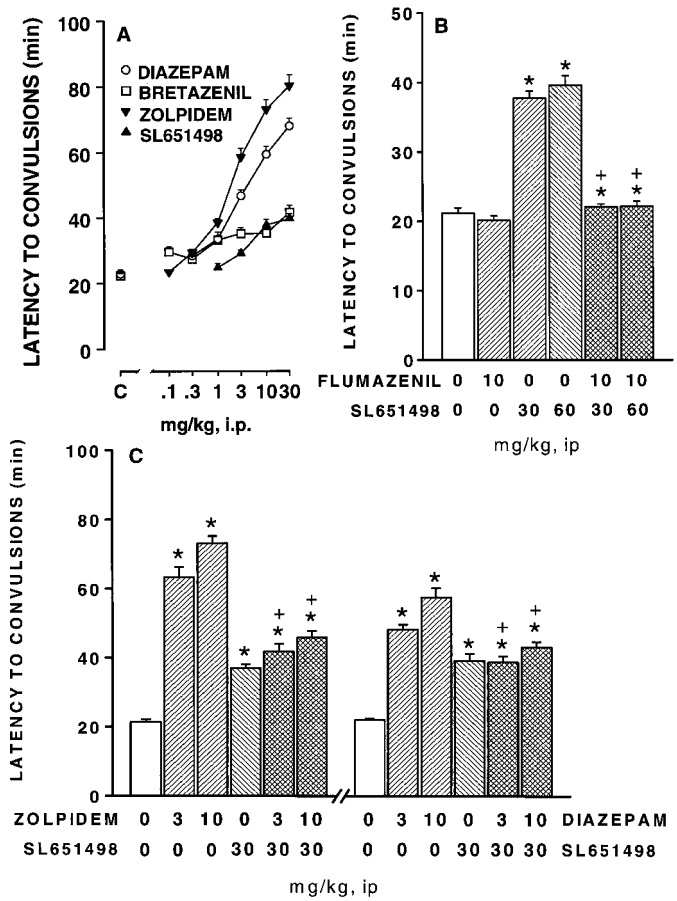


Fig. 3. A, the anticonvulsant effects of SL651498, diazepam, bretazenil, and zolpidem against clonic seizures produced in mice by 800 mg/kg s.c. isoniazid. Data of bretazenil and zolpidem are from Griebel et al. (1999a,b). $^*P < 0.05$ (Dunnett's t test). B, antagonism by flumazenil of the anticonvulsant effects of SL651498 against isoniazid. $^*P < 0.05$ (versus control, Newman-Keuls test), $^+P < 0.05$ (versus SL651498 alone). C, antagonism by SL651498 of the anticonvulsant effects of zolpidem or diazepam against isoniazid. $^*P < 0.05$ (versus control), $^+P < 0.05$ (versus zolpidem or diazepam alone) (Newman-Keuls test). Data represent mean ± S.E.M. $n = 10$.

tiated: wakefulness (W, characterized by low voltage ECoG activity), classical sleep or slow-wave sleep (SWS, characterized by an increase in ECoG activity and development of high-amplitude slow waves with some bursts of sleep spindles), and paradoxical sleep (PS) or rapid eye movement (REM) sleep (characterized by hypersynchronization of the theta rhythm in the visual area). Analysis of the ECoG signal was performed automatically by means of a computerized system discriminating between the various sleep phases using Hjorth's descriptors. Visual control was also performed.

Two types of analysis were used to quantify the effects of SL651498 on sleep-wakefulness variables: the 1-h period and the 6-h period analysis. Results are expressed in minutes (1-h period analysis) or as percentage of control values (100%). Statistical analysis was carried out using Student's t test for paired values to determine significant variations from control values.

Tolerance and Physical Dependence in Mice. Possible development of both phenomena was investigated in the isoniazid test following repeated administration of SL651498 or diazepam. Both drugs were given orally at doses of 30 or 5 mg/kg, respectively, twice daily at 8:00 AM and 4:00 PM for 10 consecutive days. These doses were chosen based on the anticonvulsant activity of the two drugs and correspond to 2 times (for diazepam) and 3 times (for SL651498) the ED_{50} against convulsions induced by isoniazid (ED_{50}, p.o. = 2.5 and 10, respectively). Forty-two hours after the last administration of the repeated treatment, mice received simultaneously a s.c. injection of isoniazid (800 mg/kg) and i.p. injections of a range of doses of SL651498 (3, 10, 30, and 100 mg/kg) or a dose of diazepam (10 mg/kg), and the latency to the first convulsion was measured as the endpoint. The effects of repeated treatment were evaluated statistically by comparing the activity of the compound observed in mice repeatedly treated with vehicle with mice repeatedly treated with drugs using two-way ANOVA followed by Newman-Keuls test.

Sensitivity of mice to isoniazid challenge was evaluated at different times (3, 6, and 14 h) after the last administration of repeated treatment. The latency to the first convulsion induced by isoniazid was measured as described above. Sensitivity was evaluated statistically by comparing minimal convulsant doses or convulsion latencies, respectively, in mice repeatedly treated with vehicle, with mice repeatedly treated with drugs, as well as those acutely receiving flumazenil using two-way ANOVA followed by Newman-Keuls test.

Interaction with Alcohol in Mice. Testing was performed using the horizontal wire test. It consisted of individually taking mice by the tail and allowing them to grasp a horizontally strung wire (20 cm above the bench level, 0.5 mm in diameter, 15 cm long) with their forepaws. Inability to grasp the wire with the forepaws or inability to actively grasp the wire within 10 s with at least one hindpaw was measured. ED_{50} values were calculated by probit analysis. Drugs were administered alone, and SL651498 or diazepam was coadministered with alcohol. Experiments were performed 60 min after oral administration of the drugs.

Drugs. In the in vivo experiments, drugs were prepared as solutions or suspensions in physiological saline containing Tween 80 (0.1%), while in the in vitro studies they were diluted in dimethyl sulfoxide that had a final concentration of 0.04%. All doses are expressed as the bases. The drugs used were SL651498, chlordiazepoxide, diazepam, flumazenil, flunitrazepam, zolpidem (synthesized by Sanofi~Synthelabo), isoniazid, pentylenetetrazole, and GABA (Sigma, St. Louis, MO). [^{3}H]Flumazenil was purchased from PerkinElmer Life Science Products (Boston, MA). Drugs administered i.p. and p.o. were given in a constant volume of 2 (rats) or 20

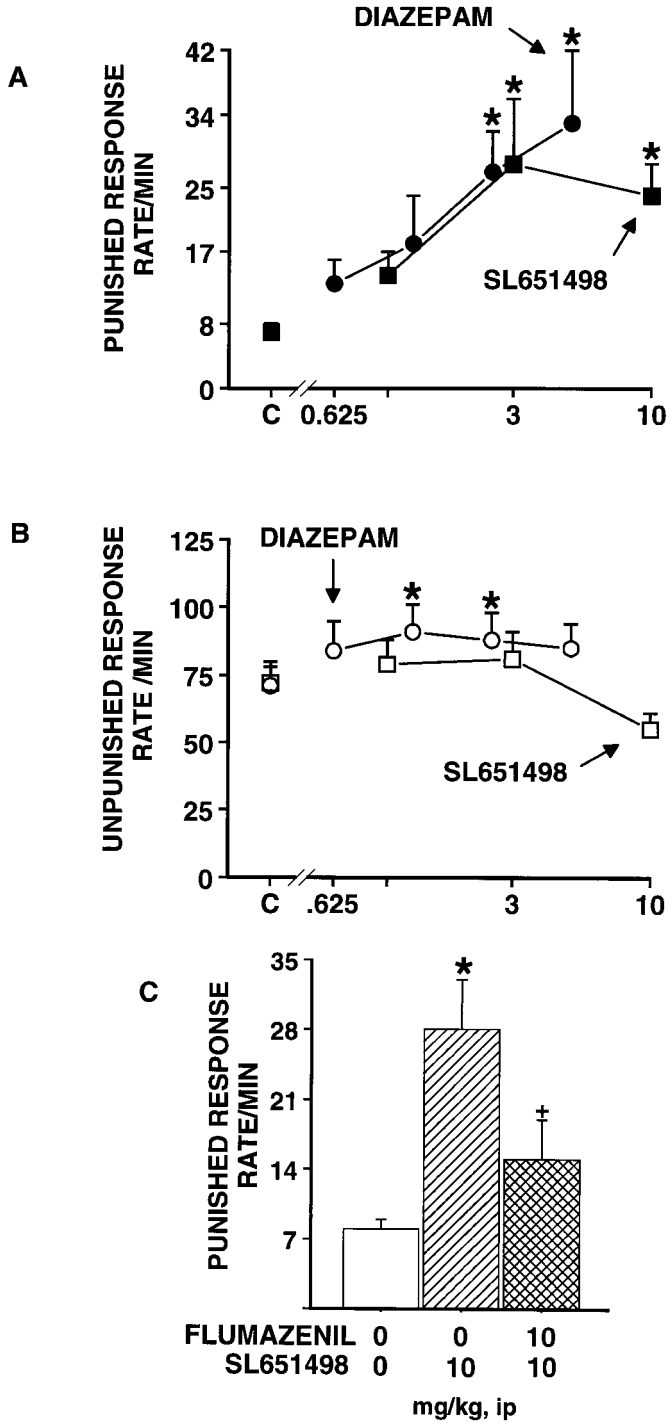


Fig. 4. Effects of SL651498 and diazepam on rates of punished (A) and unpunished (B) lever pressing in rats. C, antagonism by flumazenil of the anxiolytic-like effects of SL651498 in the punished lever pressing test. Data represent mean ± S.E.M. *$P < 0.05$ (versus control, Friedman test), $^{+}P < 0.05$ (versus SL651498 alone). $n = 8$.

(mice) ml/kg, and s.c. injections were given in a constant volume of 1 mg/kg.

Results

Biochemical and Electrophysiological Studies

[^{3}H]Flumazenil Binding to Different BZ-Sensitive $GABA_A$ Receptors in the Rat. A comparison of the affinities of diazepam and SL651498 for native and recombinant $GABA_A$ receptors containing the α_1, α_2, α_3, or α_5 subunits is shown in Table 1. Unlike diazepam, which recognized all $GABA_A$ receptor subtypes studied with nearly similar affinities, SL651498 displayed high affinity for the native α_1 (K_i = 6.8 nM) and α_2 (K_i = 12.3 nM) subtypes, but showed moderate affinity for the native α_5 (K_i = 117 nM) subtype. Essentially similar results were obtained when studying the recombinant rat $GABA_A$ receptor subtypes (K_i: $\alpha_1\beta_2\gamma_2$ = 17 nM; $\alpha_2\beta_2\gamma_2$ = 73 nM; $\alpha_3\beta_2\gamma_2$ = 80.3 nM; and $\alpha_5\beta_3\gamma_2$ = 215 nM). Differences in absolute K_i estimates for any given receptor subtype are probably due to variations in the residual amount of endogenous GABA present in these membrane preparations, but may also be due at least in part to the fact that the drugs used to selectively measure K_i values for each receptor subtype are also weakly interacting with the other receptor subtypes.

TABLE 2
Effects of SL651498 and diazepam in the punished drinking conflict test in rats
Data represent mean ± S.E.M. n = 10 to 14.

Compound	Dose	Number of Shocks
	mg/kg	
SL651498 (i.p.)	0	9.6 ± 1.2
	1	18.6 ± 4.2
	3	20.4 ± 4.5
	10	28.4 ± 4.2*
Diazepam (i.p.)	0	8.9 ± 1.3
	0.3	22 ± 4.8
	1	34.9 ± 4.8*
	3	35.5 ± 7*
	10	28.1 ± 4.2*
SL651498 (p.o.)	0	10.6 ± 1.4
	3	19.4 ± 3.3
	10	23.6 ± 3.5*
	30	21 ± 4.2
Diazepam (p.o.)	0	7.8 ± 1.7
	1	9.1 ± 3.1
	3	20 ± 5
	10	22.8 ± 4.6*
	20	25 ± 5.3*

* $P < 0.05$ (Kruskal-Wallis test).

Electrophysiological Studies. Results from these experiments showed further that SL651498 displays high activity at rat $GABA_A$ receptor subtypes. Analysis of the concentration dependence of the potentiation by SL651498 of the chloride current induced by 0.3 μM GABA $\alpha_1\beta_2\gamma_2$ cell lines showed that the maximal potentiation produced by SL651498 represents about 45% of that of the full agonist zolpidem. While at $GABA_A$ receptors expressing $\alpha_2\beta_2\gamma_2$ and $\alpha_3\beta_2\gamma_2$ subunits, the efficacy of SL651498 was comparable with that of diazepam (115 and 83% of those of diazepam, respectively), it was much lower than that of the BZ in $\alpha_5\beta_3\gamma_2$ cell lines (maximal potentiation ≈ 50% of that of diazepam) (Fig. 2). Results on the concentration dependence of the potentiation by SL651498 of the GABA (3 μM)-induced current on cultured rat DRG cells, which are enriched in α_2 subunits (Fig. 2), confirm that the drug displays similar high intrinsic efficacy as diazepam at this $GABA_A$ receptor subtype. Thus, from these data, SL651498 behaves as a full agonist at α_2- and α_3-containing $GABA_A$ receptors, and as a partial agonist at α_1- and α_5-containing $GABA_A$ receptors.

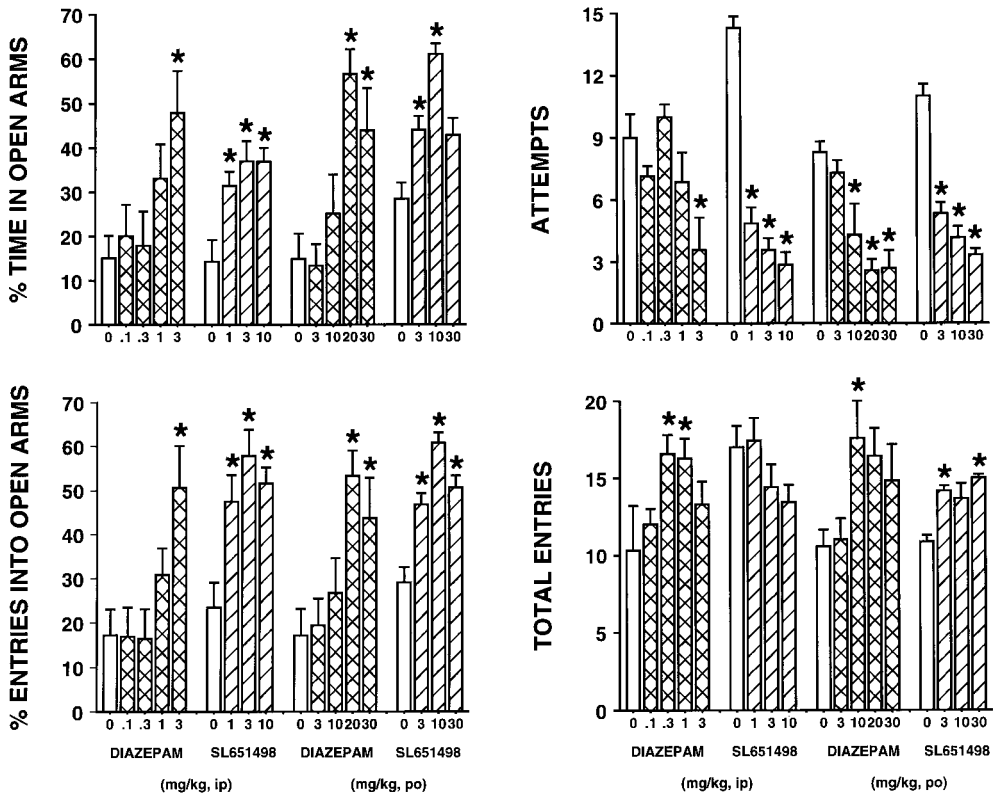


Fig. 5. Effects of SL651498 and diazepam on the behavior of rats on the elevated plus-maze. Data represent mean ± S.E.M. *$P < 0.05$ (Dunnett's t test). n = 6 to 12.

TABLE 3
Time course of the anxiolytic-like activity of SL651498 (10 mg/kg, p.o.) in the elevated plus-maze test in rats
Data represent mean ± S.E.M. n = 7 to 8.

	% Time Open Arms	% Entries Open Arms	Attempts
Control	18 ± 3.1	28.7 ± 4.4	12 ± 0.5
0 h 30 min	39.3 ± 6.5*	56.7 ± 5.7*	5.1 ± 0.9*
1 h 30 min	32.7 ± 3.1*	50.9 ± 2.6*	4.7 ± 0.8*
3 h	34.7 ± 5.3*	49.3 ± 4.1*	5.6 ± 1.3*
6 h	23 ± 4.6	39.6 ± 7.4	7.4 ± 0.9*

* $P < 0.05$ (Dunnett's t test).

Anticonvulsant Activity of SL651498

Antagonism of Pentylenetetrazole-Induced Convulsions in Mice. SL651498 and diazepam produced a dose-related increase in the number of mice protected, with ED_{50} values of 9.5 and 0.3 mg/kg (i.p.), respectively (Table 8). Maximal protection was reached at 30 mg/kg SL651498 and at 1 mg/kg diazepam.

Antagonism of Electroshock-Induced Convulsions in Mice. Diazepam but not SL651498 produced a dose-related increase in the number of mice protected, with an ED_{50} value of 1.5 mg/kg (i.p.) (Table 8). Maximal protection was reached at 6 mg/kg diazepam, while at 100 mg/kg SL651498 only 50% of the mice were protected against convulsions.

Antagonism of Isoniazid-Induced Convulsions in Mice. SL651498 [$F(5,54) = 36.99$, $P < 0.0001$], diazepam [$F(5,53) = 83.02$, $P < 0.0001$], bretazenil, and zolpidem displayed anticonvulsant activity, producing dose-related antagonism of clonic convulsions by injection of isoniazid, with ED_{50} values of 7.5, 0.7, 0.2, and 1.8 mg/kg (i.p.), respectively (Fig. 3A). However, while the intrinsic efficacy of SL651498 was similar to that of bretazenil, it was much lower than that displayed by diazepam and zolpidem. The BZ receptor antagonist flumazenil (10 mg/kg) completely blocked the anticonvulsant effects of SL651498 (30 and 60 mg/kg, i.p.) as it decreased significantly the latency to convulsions as compared with SL651498 alone [main effect: $F(5,54) = 105.5$, $P < 0.001$] (Fig. 3B). When coadministered with zolpidem or diazepam, SL651498 attenuated the anticonvulsant effects of both drugs as it shortened significantly the latency to convulsions as compared with zolpidem [main effect: $F(5,54) = 90.02$, $P < 0.0001$] or diazepam [main effect: $F(5,54) = 36.88$, $P < 0.0001$] alone (Fig. 3C).

Anxiolytic-Like Activity of SL651498

Punished Lever Pressing Test in Rats. The rates of responding decreased by the punishment contingency were significantly increased by SL651498 ($\chi^2 = 15.14$, $P < 0.01$) from 3 mg/kg, and by diazepam ($\chi^2 = 11.97$, $P < 0.0175$) at 2.5 and 5 mg/kg (i.p.) (Fig. 4A). Unpunished responding was unaffected by SL651498 and increased by diazepam ($\chi^2 = 10.9$, $P < 0.0277$) at 1.25 and 2.5 mg/kg (Fig. 4B). The anxiolytic-like effects of SL651498 (10 mg/kg, i.p.) in this model were completely antagonized by the BZ receptor antagonist flumazenil (10 mg/kg) (main effect: $\chi^2 = 7$, $P < 0.05$) (Fig. 4C).

Punished Drinking Test in Rats. SL651498 (i.p.: KW = 9.16, $P < 0.05$; p.o.: KW = 7.86, $P < 0.05$) and diazepam (i.p.: KW = 9.24, $P < 0.05$; p.o.: KW = 15.61, $P < 0.01$) significantly increased punished responding at 10 mg/kg, whereas diazepam produced similar effects from 1 and 10 mg/kg after i.p. and p.o. administration, respectively (Table 2).

Elevated Plus-Maze Test in Rats. SL651498 and diazepam modified all anxiety-related measures (Fig. 5). All drug treatments significantly modified both the percentage of time spent [i.p.: SL651498: $F(3,24) = 7.11$, $P < 0.01$; diazepam:

TABLE 4
Effects of SL651498 and diazepam on the behavior of mice in the light/dark test
Data represent mean ± S.E.M. $n = 12$.

Compound	Dose	Time in the Lit Box(s)	Number of Attempts	Tunnel Crossings
	mg/kg, i.p.			
SL651498	0	7.1 ± 6.2	21.1 ± 1.7	2.4 ± 1
	1	9.8 ± 7.8	19.4 ± 1.6	2.6 ± 1.4
	3	18.4 ± 10.6	18.2 ± 2.9	3.8 ± 1.2
	10	28.9 ± 8.5*	12.1 ± 1.7*	5.9 ± 1.3
Diazepam	0	0 ± 0	13.5 ± 1.9	1 ± 0
	0.5	0 ± 0	15.9 ± 2.6	1 ± 0
	1	10.2 ± 4.6	20.3 ± 1.5	4.3 ± 1.2
	2	78.3 ± 15.3*	9.5 ± 2.1	12.8 ± 1.9*
	4	113 ± 17.9*	5.3 ± 1.9*	10.6 ± 1.8*
	8	110.4 ± 18.8*	3.3 ± 0.7*	7.4 ± 1.3*

* $P < 0.05$ (Kruskal-Wallis test).

TABLE 5
Effects of SL651498 and diazepam on the behavior of mice in the four-plate test
Data represent mean ± S.E.M. $n = 10$.

Compound	Dose	Punished Crossings
	mg/kg	
SL651498 (i.p.)	0	5.9 ± 0.4
	2	8.4 ± 0.9
	4	9.8 ± 0.8*
	8	8.6 ± 0.8*
Diazepam (p.o.)	0	6.8 ± 0.7
	2	14 ± 1.4*
	4	18.7 ± 1.1*
	8	13.3 ± 1.6*

* $P < 0.05$ (Dunnett's t test).

$F(4,30) = 3.27$, $P < 0.05$; p.o.: SL651498: $F(3,21) = 6.58$, $P < 0.001$; diazepam: $F(4,29) = 7.37$, $P < 0.001$] and the percentage of entries made [i.p.: SL651498: $F(3,24) = 7.79$, $P < 0.001$; diazepam: $F(4,30) = 4.39$, $P < 0.01$; p.o.: SL651498: $F(3,21) = 8.34$, $P < 0.001$; diazepam: $F(4,29) = 5.21$, $P < 0.01$] into open arms. Post hoc analysis indicated that SL651498 (i.p. and p.o.) and diazepam (p.o.) significantly increased activity in open arms at several doses, whereas i.p. administered diazepam produced a significant increase in open-arm time and entries at the highest doses only (3 mg/kg). With respect to the ethologically derivated measure, all treatments modified the number of attempts at entry into open arms followed by avoidance responses [i.p.: SL651498: $F(3,24) = 71.29$, $P < 0.001$; diazepam: $F(4,30) = 4.74$, $P < 0.01$; p.o.: SL651498: $F(3,21) = 26.44$, $P < 0.001$; diazepam: $F(4,29) = 8.73$, $P < 0.001$]. Post hoc analysis showed that SL651498 (i.p. and p.o.) and diazepam (p.o.) significantly reduced attempts at several doses, whereas i.p. diazepam decreased this behavior at the highest dose only (3 mg/kg). Finally, SL651498 [p.o.: $F(3,21) = 4.21$, $P < 0.05$] and diazepam [i.p.: $F(4,30) = 4.89$, $P < 0.01$; p.o.: $F(4,29) = 2.98$, $P < 0.05$] significantly increased the number of total arm entries. These effects reached statistical significance with SL651498 at 3 and 30 mg/kg (p.o.), and with diazepam at 0.3 and 1 (i.p.) and at 10 mg/kg (p.o.). The time course of the anxiolytic-like action of orally administered SL651498 at 10 mg/kg indicated that effects lasted up to 3 h as measured on both spatio-temporal parameters [main effects: percentage of open-arm time: $F(4,35) = 4.4$, $P < 0.01$, and entries: $F(4,35) = 5.5$, $P < 0.01$], and up to 6 h with respect to attempts [main effect: $F(4,35) = 16.2$, $P < 0.001$] (Table 3).

Light/Dark Test in Mice. Diazepam (KW = 48.76, $P < 0.001$) at 2 to 8 mg/kg (i.p.) and SL651498 (KW = 9.79, $P < 0.05$) at 10 mg/kg (i.p.) significantly increased time spent by mice in the lit box, although the magnitude of the effects of SL651498 was less than that of diazepam (Table 4). The number of attempts at entry into the lit box was significantly reduced by diazepam (4 and 8 mg/kg) (KW = 35.15, $P < 0.001$) and by SL651498 (10 mg/kg) (KW = 11.04, $P < 0.05$). Finally, diazepam (2–8 mg/kg) (KW = 44.6, $P < 0.001$), but not SL651498, significantly increased tunnel crossings.

Four-Plate Test in Mice. Diazepam [$F(3,44) = 15.61$, $P < 0.01$] at all doses (from 2–8 mg/kg, p.o.) and SL651498 [$F(3,36) = 4.8$, $P < 0.01$] from 4 mg/kg (i.p.) significantly increased the number of punished crossings (Table 5); however, the magnitude of the effects of SL651498 was less than that of diazepam.

Mouse Defense Test Battery (Table 6). (a) *Pretest: Motor activity before exposure to the rat:* Statistical analyses revealed that neither acute nor chronic SL651498 treatment significantly modified the number of line crossings at the doses tested. In contrast, diazepam significantly decreased line crossings at 10 and 20 mg/kg [$F(4,45) = 7.76$, $P < 0.001$] after an acute oral administration; (b) *Chase/flight test:* After acute administration, both drugs significantly modified the number of orientations [SL651498: i.p.: $F(3,44) = 3.02$, $P < 0.05$; p.o.: $F(3,32) = 14.05$, $P < 0.001$; diazepam: i.p.: $F(3,28) = 9.4$, $P < 0.001$; p.o.: $F(4,45) = 37.53$, $P < 0.001$] and the number of stops [SL651498: i.p.: $F(3,44) = 4.46$, $P < 0.01$; p.o.: $F(3,32) = 17.31$, $P < 0.001$; diazepam: i.p.: $F(3,28) = 14.52$, $P < 0.001$; $F(4,45) = 50.53$, $P < 0.001$]. Post hoc analysis revealed that SL651498 significantly reduced orientations at 10 (i.p.) and from 3 (p.o.) mg/kg, and stops at 10 (i.p.) and from 10 (p.o.) mg/kg. Diazepam significantly reduced orientations from 1 mg/kg (i.p. and p.o.), and stops from 1 (i.p.) and 3 (p.o.) mg/kg. In the repeated treatment experiments, ANOVA revealed a significant main effect for orientations [$F(8,63) = 3.71$, $P < 0.01$] and stops [$F(8,63) = 4.79$, $P < 0.001$]. Post hoc analysis showed that SL651498 significantly decreased both measures in mice treated repeatedly by saline and then challenged by a single dose of the drug (10 mg/kg). Following repeated administration of SL651498, orientations were significantly decreased from 3 mg/kg, and stops at 3 mg/kg. In the case of diazepam, these

TABLE 6

Effects of acute and repeated (twice per day for 13 days) treatments with diazepam and SL651498 on several behavioral responses displayed by Swiss mice before (locomotor activity), during (risk assessment and defensive attack), and after (contextual defense) exposure to a Long Evans rat in the mouse defense test battery

Data represent mean ± SEM. n = 8 to 12.

	Dose	Locomotor Activity	Risk Assessment			Defensive Attack	Contextual Defense
		Line Crossings	Stops	Orientations	Approaches-Withdrawals	Bitings	Escape Attempts
	mg/kg						
Acute Experiments							
SL651498 (i.p.)	0	107.3 ± 12.5	11.6 ± 1.6	8.9 ± 1.8	1.1 ± 0.2	2.5 ± 0.3	35.1 ± 2
	1	137 ± 6	11.1 ± 1.4	8.2 ± 1.4	1.3 ± 0.4	2.7 ± 0.1	30.5 ± 2.3
	3	143.5 ± 12.5	11.8 ± 2	9.8 ± 1.8	1.5 ± 0.3	2.3 ± 0.3	26.9 ± 2.8
	10	121.6 ± 8.2	5.2 ± 1.2*	4 ± 1.2*	1.5 ± 0.4	0.8 ± 0.3*	11.8 ± 2.2*
Diazepam (i.p.)	0	127.6 ± 9.6	13.3 ± 2	9.6 ± 1.9	0 ± 0	2.3 ± 0.3	30.6 ± 3.8
	0.5	138.6 ± 16.1	10.1 ± 1.4	5.9 ± 1.5	0.3 ± 0.2	1.8 ± 0.2	27.3 ± 4.9
	1	139.5 ± 22	3.6 ± 0.6*	2.3 ± 0.6*	0.8 ± 0.3	0.8 ± 0.2*	24.5 ± 5.4
	3	95.1 ± 13.7	3.1 ± 0.8*	1.3 ± 0.4*	1.6 ± 0.6*	0.1 ± 0.1*	19 ± 5.8
SL651498 (p.o.)	0	123.6 ± 10	9.8 ± 0.2	6.7 ± 1.1	0 ± 0	2.6 ± 0.2	35.2 ± 11.7
	3	120.9 ± 9	8.9 ± 0.2	5.4 ± 0.4*	0 ± 0	2 ± 0.2*	32.6 ± 10.9
	10	111.7 ± 8	6.2 ± 0.4*	3 ± 0.3*	0.1 ± 0.1	0.1 ± 0.1*	17.4 ± 5.8*
	30	99.7 ± 10.9	5 ± 0.6*	1.8 ± 0.4*	0.6 ± 0.3*	0.1 ± 0.1*	16.1 ± 5.4*
Diazepam (p.o.)	0	153.3 ± 8.2	9.3 ± 0.2	6.9 ± 0.5	0.1 ± 0.1	2.6 ± 0.2	37.2 ± 3.7
	1	153.3 ± 9.2	8.8 ± 0.3	5.5 ± 0.5*	0.2 ± 0.1	2.2 ± 0.3	29.7 ± 2.9
	3	136.6 ± 7.2	6.6 ± 0.3*	3.3 ± 0.5*	0.7 ± 0.3	0.3 ± 0.2*	18.8 ± 3.5*
	10	111.3 ± 4.8*	5.2 ± 0.3*	1.4 ± 0.3*	2.3 ± 0.4*	0 ± 0*	3.2 ± 1.2*
	20	111.1 ± 8*	4.8 ± 0.4*	1.3 ± 0.3*	2.6 ± 0.5*	0 ± 0*	4.2 ± 2.2*
Repeated Experiments							
SL651498 (acute, p.o.)	0	116.6 ± 10.3	6.8 ± 1.2	9.3 ± 0.8	0.1 ± 0.1	2.5 ± 0.2	30.1 ± 5.4
	1	103.6 ± 10.2	5.6 ± 1	8.3 ± 1	0.9 ± 0.4	1.4 ± 0.3	26.8 ± 3.2
	3	122.4 ± 12.9	4.8 ± 1.1	6.8 ± 1	0.8 ± 0.3	1.3 ± 0.4*	23 ± 3.2
	10	110.0 ± 8.7	2.1 ± 0.5*	4.4 ± 0.6*	0.5 ± 0.3	0.3 ± 0.2*	21.3 ± 3.9
Diazepam (acute, p.o.)	3	94.1 ± 7.2	1.6 ± 0.6*	3.4 ± 0.7*	1.5 ± 0.5	0 ± 0*	16 ± 4.6
SL651498 (repeated, p.o.)	1	90.9 ± 10.8	4.4 ± 0.9	6.9 ± 0.7	0.4 ± 0.2	1.8 ± 0.4	24.9 ± 2.7
	3	119.4 ± 9.1	3.8 ± 0.7*	5.4 ± 0.9*	1 ± 0.4	0.8 ± 0.5*	22.9 ± 5
	10	103.9 ± 5.3	4.4 ± 0.8	6.5 ± 1*	1.3 ± 0.4	0 ± 0*	24.1 ± 3.4
Diazepam (repeated, p.o.)	3	104 ± 11.5	3.4 ± 0.6*	5.1 ± 0.7*	1.4 ± 0.4	0 ± 0*	20.9 ± 3.5

* $P < 0.05$ (relative to zero drug treatment, Dunnett's t test).

behaviors were significantly reduced at 3 mg/kg, following both acute and repeated treatments; (c) *Straight alley test:* In the acute experiments, diazepam but not SL651498 significantly modified the number of approaches to the rat followed by withdrawal responses after i.p. administration [$F(3,28) = 4.63$, $P < 0.01$]. Post hoc analysis showed that the BZ significantly increased this behavior at 3 mg/kg. Following oral administration, SL651498 [$F(3,32) = 3.57$, $P < 0.05$] and diazepam [$F(4,45) = 13.44$, $P < 0.001$] significantly increased this response at 30 mg/kg and from 10 mg/kg, respectively. In the repeated treatment experiment, ANOVA just failed to reach statistical significance ($P = 0.065$), but diazepam (3 mg/kg) after both regimens, and SL651498 (10 mg/kg) administered repeatedly, increased approach/withdrawal behavior; (d) *Forced contact test:* After both regimens, SL651498 [acute: i.p.: $F(3,44) = 11.25$, $P < 0.001$; p.o.: $F(3,32) = 58.19$, $P < 0.001$; chronic: $F(8,63) = 10.81$, $P < 0.001$] and diazepam [i.p.: $F(3,28) = 27.96$, $P < 0.001$; p.o.: $F(4,45) = 60.32$, $P < 0.001$] significantly modified defensive biting upon forced contact with the rat. Post hoc analysis indicated that in the acute treatment experiments, SL651498 significantly reduced defensive attack at 10 (i.p.) and from 3 (p.o.) mg/kg, and diazepam at 1 (i.p.) and from 3 (p.o.) mg/kg. In the repeated treatment experiment, biting was reduced following both single and repeated administrations of SL651498 (acute and repeated: 3 and 10 mg/kg) and diazepam (acute and repeated: 3 mg/kg); (e) *Post-test escape attempts:* After acute i.p. injection, SL651498 [$F(3,44) = 18.6$, $P < 0.001$], but not diazepam, significantly decreased the number of escape attempts following the removal of the rat from the runway apparatus (10 mg/kg). Following oral administration, SL651498 (10 and 30 mg/kg) and diazepam (3–20 mg/kg) significantly decreased this behavior [SL651498: $F(3,32) = 20.22$, $P < 0.001$; diazepam: $F(4,45) = 29.02$, $P < 0.001$]. In the repeated treatment experiment, neither drug significantly modified escape attempts.

Discriminative Stimulus Properties in Rats

The effects of SL651498 in rats trained to discriminate a dose of 5 mg/kg chlordiazepoxide or a dose of 2 mg/kg (i.p.) zolpidem from saline are shown in Fig. 6. SL651498 fully generalized to chlordiazepoxide at 10 mg/kg (i.p.), but only partially substituted for zolpidem at 30 mg/kg (i.p.). Similar differences between the two discrimination cues were observed with diazepam. SL651498 was as potent as chlordiazepoxide in producing generalization as indicated by the ED_{50} values (1.7 versus 2.2 mg/kg).

Side Effect Profile of SL651498

Rotarod Test in Mice and Rats. In both species, SL651498 [mice: $F(4,35) = 2.66$, $P < 0.05$; rats: $F(5,36) = 15.41$, $P < 0.001$] and diazepam [mice: $F(4,45) = 27.95$, $P < 0.001$; rats: $F(5,36) = 9.41$, $P < 0.001$] significantly impaired rotarod performance. Subsequent analysis showed that diazepam significantly reduced the time on the rotarod at 10 and 30 mg/kg (i.p.), whereas SL651498 decreased this measure at

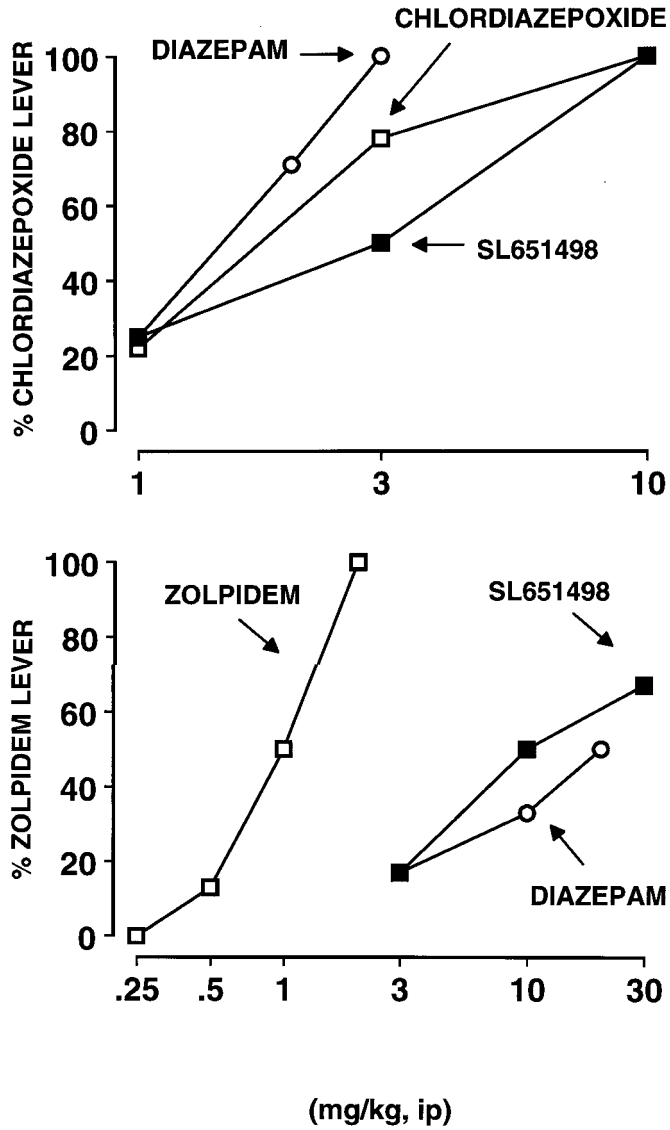


Fig. 6. Effects of SL651498, chlordiazepoxide, diazepam, or zolpidem in rats trained to discriminate chlordiazepoxide (5 mg/kg) (top) or zolpidem (2 mg/kg) (bottom) from saline. The results are shown as the percentage of animals responding on the chlordiazepoxide or zolpidem lever. $n = 6$ to 9.

100 mg/kg (i.p.) in mice and from 30 mg/kg (i.p.) in rats (Fig. 7A). Calculated ED_{50} values for diazepam were 10.1 (mice) and 6.5 (rats) mg/kg, while that of SL651498 in rats was 37 mg/kg. The ED_{50} value for SL651498 in mice could not be determined (>100 mg/kg, i.p.).

Grip Strength Test in Mice and Rats. In mice, diazepam, but not SL651498, markedly decreased forelimb grip strength scores with ED_{50} values of 3.5 and 56 mg/kg (i.p.), respectively (Fig. 7B). Similarly, in rats, the BZ dramatically reduced this measure at low doses (ED_{50} = 2.9 mg/kg, i.p.), whereas SL651498 produced deficit in performance at the highest doses only (ED_{50} = 27 mg/kg, i.p.) (Fig. 7B).

ECoG Studies in Rats. SL651498 did not induce any significant change of the sequential spectral analysis up to 30 mg/kg (i.p. or p.o.). At this dose, slight modification of the EEG pattern occurred corresponding to drowsiness. In contrast, diazepam (i.p.) induced a dose-dependent concomitant increase of slow and fast waves as well as bursts of sleep spindles of 12 to 14 Hz, a pattern classically seen with BZs. (Fig. 8). The quantified EEG spectral analysis showed that SL651498 induced a slight increase of the total power from 30 mg/kg (p.o. or i.p.) (Table 7A). This was in contrast to diazepam, which produced a marked increase of the total power from 1 mg/kg (i.p.). At 3 mg/kg of diazepam, the total power further increased to reach almost 200% of control values. The quantified EEG spectral analysis for each frequency band in the SM cortex after SL651498 showed only weak increases of the energy in the delta, alpha, and beta frequency bands from 30 mg/kg (i.p.). Diazepam increased dramatically the energy in the delta, alpha, and beta frequency bands from 1 mg/kg (i.p.).

Sleep-Wakefulness Cycle Studies in Chronically Implanted Rats. The effects of SL651498 (10 mg/kg, p.o.) on sleep-wakefulness parameters are summarized in Table 7B. The drug did not significantly disrupt the pattern of the sleep-wakefulness cycle in chronically implanted rats, nor did it enhance slow wave sleep or alter paradoxical sleep, indicating the absence of sedative effect and modification of the sleep architecture.

Tolerance in Mice. The potency of SL651498 in antagonizing isoniazid-induced convulsions was not significantly changed after its repeated administration for ten days (b.i.d.) [drug × pretreatment interaction: $F(4,90) = 2.31$, $P > 0.05$] (Fig. 9A). After repeated administration, however, diazepam was significantly less active in increasing the latency to convulsions [drug × pretreatment interaction: $F(1,36) = 13.66$, $P < 0.001$] (Fig. 9A).

Physical Dependence in Mice. After discontinuation of the treatment with SL651498, significant increases in latencies to convulsions were observed 3 h after the last administration [drug × pretreatment interaction: $F(7,126) = 3.17$, $P < 0.01$] (Fig. 9B). Six and 14 h after the last administration of SL651498, the latencies to convulsions were again similar to those observed in mice repeatedly treated with vehicle. After discontinuation of repeated administration with diazepam, significant increases in latencies to convulsions were observed 3 and 6 h after the last administration [drug × pretreatment interaction: $F(6,117) = 10.1$, $P < 0.001$] (Fig. 9C). This anticonvulsant effect was not present 14 h after discontinuation of the treatment. When flumazenil was given 3, 6, and 14 h after the last administration of diazepam, but not of SL651498, the anticonvulsant effect of the BZ was reversed and the latency to convulsions was decreased as compared with latencies observed in mice repeatedly treated with vehicle.

Interaction with Alcohol in Mice. When administered alone, alcohol and SL651498 produced minimal effects on motor performance in the horizontal wire test ($ED_{50} > 1.6$ g and 30 mg/kg, respectively), whereas diazepam produced impairment at 3 mg/kg (ED_{50} = 4.1 mg/kg). While SL651498 potentiated the effects of alcohol at the highest dose only (30 mg/kg), diazepam showed an additive effect with alcohol from 1 mg/kg (data not shown).

Discussion

The in vitro binding experiments to different $GABA_A$ receptor subtypes showed that SL651498 and diazepam displaced [^{3}H]flumazenil binding in rat brain areas enriched in α_1-, α_2-, and α_3-containing $GABA_A$ receptor subtypes, or their

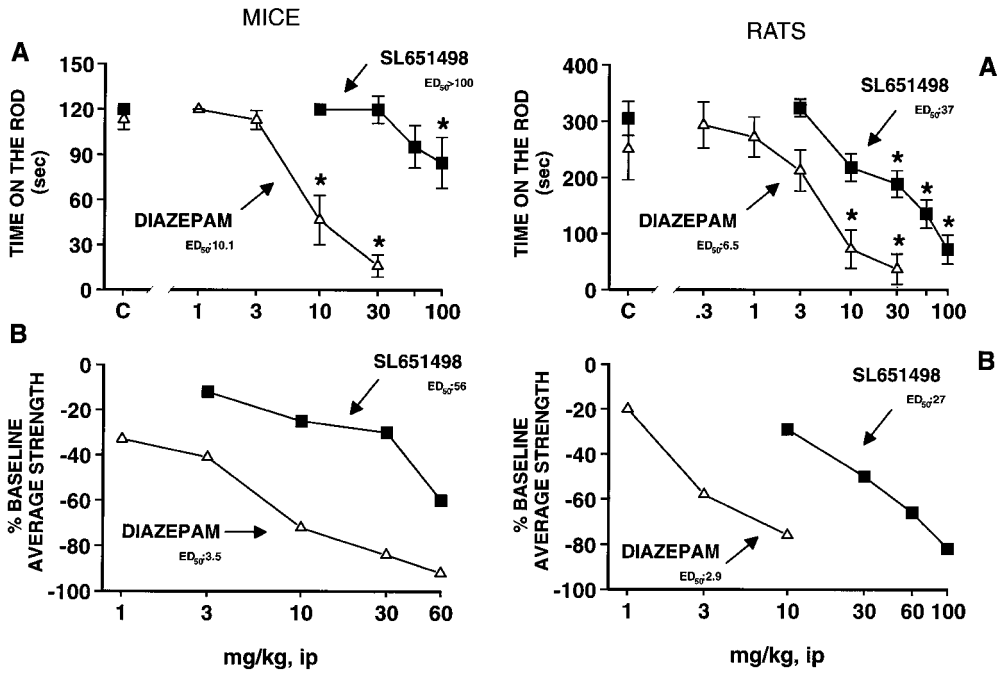


Fig. 7. Effects of SL651498 and diazepam on motor performance in mice and rats in the rotarod (A) and the grip strength (B) tests. $^*P < 0.05$ (Dunnett's t test). Data represent mean ± S.E.M. (rotarod) or percentage variation from baseline levels (grip strength). $n = 6$ to 10.

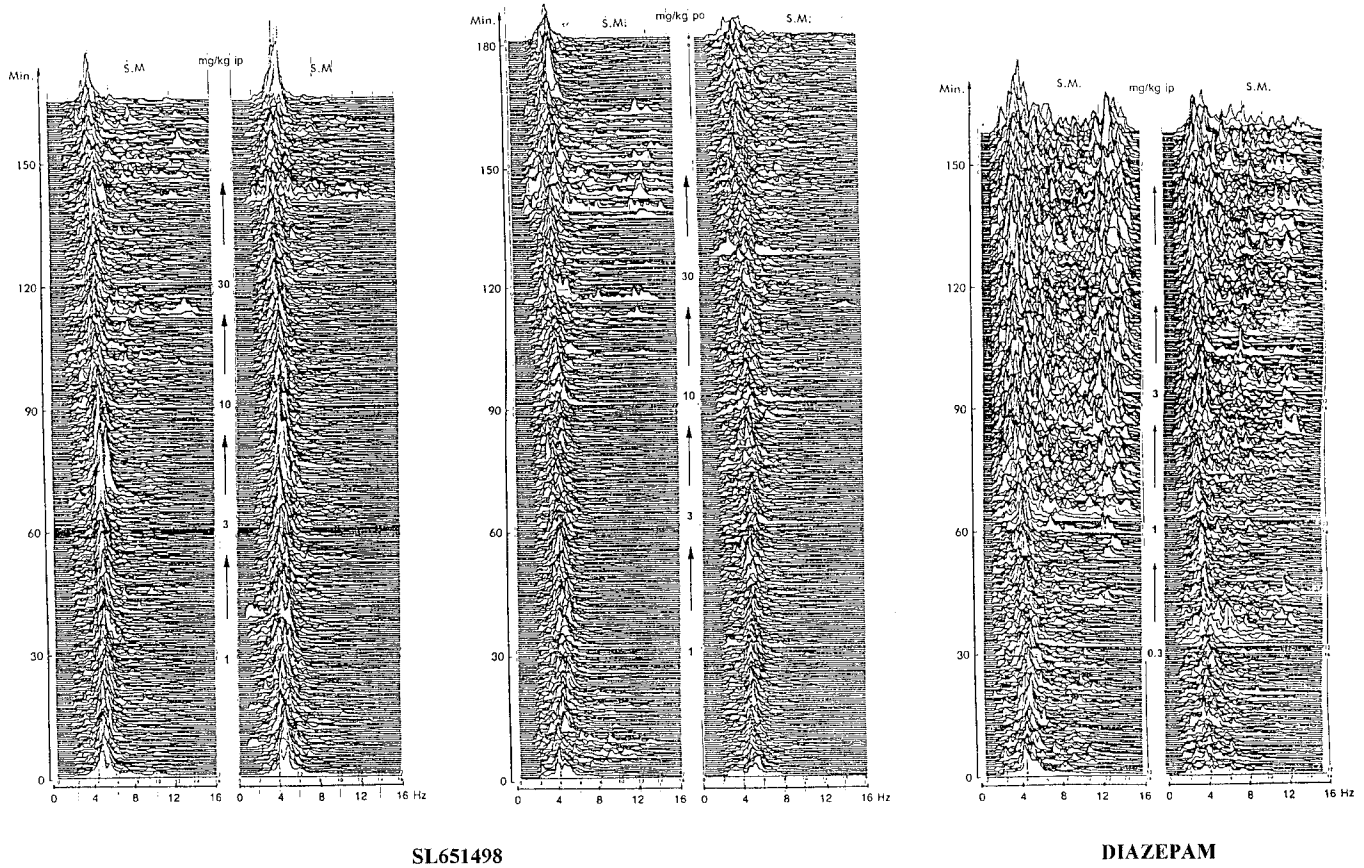


Fig. 8. Examples of three-dimensional sequential spectral analysis in the SM cortex of rats before and after administration of increasing doses of SL651498 (i.p. and p.o.) and diazepam (i.p.). The left and middle panels represent the profile of individual rats (two per panel) administered with i.p. and p.o. doses of SL651498, respectively, and the right panel represents two rats injected with i.p. doses of diazepam. Baseline EEG activity was recorded for 30 min before the drugs were administered at increasing doses every 30 min. Five frequency bands are shown on this figure: delta (1–4 Hz), theta (4.5–7 Hz), alpha 1 (7.5–9.5 Hz), alpha 2 (10–12.5 Hz), and beta (>13 Hz).

recombinant counterparts, with approximately the same affinities. However, in contrast to diazepam, SL651498 displayed only moderate potencies at the native or recombinant α_5-containing $GABA_A$ receptor subtype in the hippocampus. Furthermore, while in transfected $GABA_A$ receptors expressing $\alpha_2\beta_2\gamma_2$ and $\alpha_3\beta_2\gamma_2$ subunits, and in native receptors of rat

TABLE 7

A, quantitative EEG spectral analysis in the rat sensorimotor cortex: effects of SL651498 and diazepam. Statistically significant increases (% variations) of absolute percent of total power values for each frequency band and total power after increasing doses of SL651498 (3, 10, and 30 mg/kg) or diazepam (0.3, 1 and 3 mg/kg) in rats; B, effects of SL651498 on sleep-wakefulness cycle in rats recorded during the dark period: global analysis of a 6-h period. $n = 6$.

A

Compound	Dose	Delta Band	Theta Band	Alpha 1 Band	Alpha 2 Band	Beta 1 Band	Beta 2 Band	Total Power
	mg/kg							
SL651498 (p.o.)	10	N.S.	N.S.	N.S.	N.S.	N.S.	+37 to +46	
	30	+66 to +90	N.S.	+82 to +128	+143 to +189	+105 to +158	+46 to +91	+30 to +56%
SL651498 (i.p.)	10	+37 to +56	N.S.	N.S.	N.S.	N.S.	N.S.	
	30	+67 to +90	N.S.	+51 to +97	+70 to +107	+52 to +94	+30 to +39	+58 to +79%
Diazepam (i.p.)	1	+82 to +149	N.S.	+127 to +145	+155 to +216	+198 to +270	+167 to +261	+93 to +126%
	3	+115 to +210	+50 to +72	+116 to +241	+178 to +334	+255 to +450	+167 to +261	+115 to 202%

B

SL651498	W%	SWS%	PS%	nPS%	xPS%	PS lat
						min
10 mg/kg, p.o.	0	+3	−27	−29	−5	46
24 h after	+6	−11	−26	−29	+5	100

N.S., not significant; W, wakefulness; SWS, slow wave sleep; PS, paradoxical sleep; nPS, number of PS phases; xPS, mean duration of PS phases; PS lat, delay of the onset of the first PS phase.

* $P < 0.05$ (Student's t test for paired values).

DRG neurons, a region enriched in the α_2 subtype, the efficacy of SL651498 reached that of diazepam, it was much lower than that of zolpidem and diazepam, in $\alpha_1\beta_2\gamma_2$ and $\alpha_5\beta_3\gamma_2$ subunits. Thus, SL651498 behaves as a full agonist at the $GABA_A$ receptor α_2 and α_3 subtypes and as a partial agonist at the $GABA_A$ receptor α_1 and α_5 subtypes.

SL651498 antagonized both the tonic and clonic convulsions induced either by various chemical convulsants or by maximal electroshock, as did diazepam. However, unlike this BZ, which antagonized convulsions with similar potencies irrespective of the nature of the convulsant challenge, SL651498 was more potent against pentylenetetrazole than against electroshock. This anticonvulsant profile is consistent with a partial agonist activity (bretazenil) or preferential affinity (abecarnil, zolpidem) for α_1-containing $GABA_A$ receptors (Martin et al., 1988; Perrault et al., 1990; Turski et al., 1990). In addition, diazepam produced a greater delay in onset of isoniazid-induced convulsions than SL651498. The maximal delay in onset of isoniazid-induced seizures produced by a test compound has been proposed as an in vivo measure of the intrinsic activity of GABA/BZ receptor ligands at the $GABA_A$ receptor α_1 subtype (Mao et al., 1975). The observation that SL651498 displayed similar low intrinsic efficacy as bretazenil, together with the finding that the former antagonized the anti-isoniazid effects of diazepam and zolpidem, strengthens the electrophysiological data showing that SL651498 behaves as a partial agonist at $GABA_A$ receptor subtypes expressing α_1 subunits.

Results from anxiety tests showed that SL651498 produced anxiolytic-like effects in a variety of experimental procedures. It increased rates of responding suppressed by punishment in the punished lever pressing and the punished drinking conflict tests in rats, and in the four-plate test in mice. The behavioral profile of SL651498 in exploration models of anxiety showed that it produced significant effects on all anxiety-related measures in the rat elevated plus-maze and the mouse light/dark tests. The finding that SL651498, unlike diazepam, did not stimulate tunnel crossings in the light/dark test and only weakly increased total arm entries in the elevated plus-maze suggests that the drug may have fewer disinhibitory properties than BZs. An additional experiment showed that the anxiolytic-like activity of orally administered SL651498 (10 mg/kg) lasted up to 3 h in the elevated plus-maze. In the mouse defense test battery, SL651498 and diazepam markedly modified defensive behaviors. Prominent effects of the drugs were observed on risk assessment activities and defensive attack reactions. All these effects are consistent with an anxiolytic-like action in this test (Griebel et al., 1995). After repeated administration, the effects of SL651498 were still evident, indicating that no tolerance to the anxiolytic-like activity has developed. Instead, repeated administration of SL651498 tended to potentiate its effects as was evidenced by the lower minimal effective dose following this treatment (i.e., 3 versus 10 mg/kg after acute treatment) on some risk assessment measures.

The finding that the effects of SL651498 in the punished lever pressing test were antagonized by the nonselective $GABA_A$ receptor antagonist flumazenil indicates that central BZ receptors are involved in the anxiolytic-like action of SL651498. However, it is not clear whether these effects involve all or only certain $GABA_A$ receptor subtypes. The weak affinity of SL651498 at the $GABA_A$ receptor α_5 subtype would suggest that this receptor subtype is not responsible for the anxiolytic-like action of the drug. Furthermore, in the drug discrimination experiments, the internal stimulus produced by SL651498 was found to substitute completely for the chlordiazepoxide anxiolytic-like cue, whereas it produced only partial substitution for the zolpidem α_1-dependent sedative cue (Sanger and Zivkovic, 1986; Cooper et al., 1987; Sanger et al., 1999), suggesting that $GABA_A$ receptor subtypes other than those bearing the α_1 subunit were involved in these effects. Moreover, the sedative effects and weak (if any) anxiolytic-like activity displayed by $GABA_A$ receptor agonists that display preferential affinity for the α_1 subtype (abecarnil, RWJ-46771, SX-3228, zaleplon, zolpidem) in previous studies suggest that this subtype is primarily associated with sedation and may not be involved in the anxiolytic-like effects of GABA/BZ receptor agonists (Zivkovic et al., 1992; Sanger, 1995; Griebel et al., 1999b). This idea is further strengthened by recent studies using mice with point-mu-

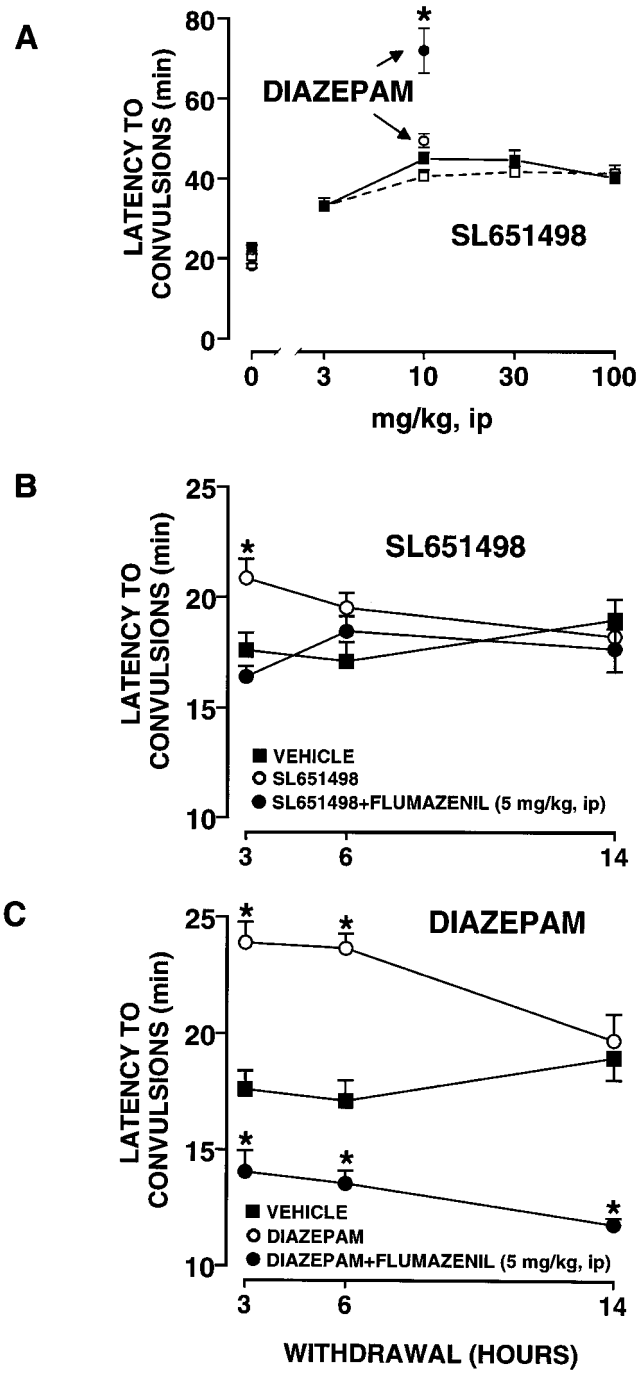


Fig. 9. A, effects of repeated treatment with SL651498 or diazepam on the potency of these drugs to increase the latency to convulsions induced by isoniazid. SL651498 or diazepam was given simultaneously with isoniazid (800 mg/kg, s.c.) 42 h after the last administration of the repeated treatment with SL651498 (2 × 30 mg/kg, p.o.) or diazepam (2 × 5 mg/kg, p.o.) for 10 consecutive days. Data represent mean ± S.E.M. in mice repeatedly treated with vehicle (filled symbols), SL651498, or diazepam (open symbols). $^*P < 0.05$ (versus repeated vehicle, Newman-Keuls test). B and C, effects of repeated treatment with SL651498 (2 × 30 mg/kg, p.o.) or diazepam (2 × 5 mg/kg, p.o.) for 10 consecutive days on the sensitivity of mice to the convulsant effect of isoniazid. The time to the appearance of the first convulsion induced by isoniazid (800 mg/kg, s.c.) was noted at different times after the last administration of chronic SL651498, diazepam, or vehicle. Data represent mean ± S.E.M. in mice repeatedly treated with vehicle (■), SL651498 or diazepam (○), or mice repeatedly treated with SL651498 or diazepam and which received an injection of flumazenil 10 min after administration of isoniazid (●). $^*P < 0.05$ (versus repeated vehicle, Newman-Keuls test). $n = 10$.

tated diazepam or zolpidem-insensitive $GABA_A$ receptor subtypes, which showed that the anxiolytic-like action of diazepam is absent in animals with the α_2 but not the α_1 or α_3 point mutation, whereas the sedative effects of zolpidem are lost in mice with the α_1 point mutation (Rudolph et al., 1999; Crestani et al., 2000; Low et al., 2000). Together, these findings strongly suggest that the anxiolytic-like effects of SL651498 may involve primarily the α_2-containing $GABA_A$ receptor subtype upon which the drug acts as full agonist.

SL651498 produced motor impairment at higher doses than those producing anxiolytic-like effects. While this may also be the case with diazepam and two other BZs (i.e., clorazepate and lorazepam, see Table 8) tested previously under similar experimental conditions, on average, the therapeutic ratio of SL651498 compares favorably with those of these BZs. For example, the EEG analysis showed that SL651498 induced slight sedative effects at a dose (30 mg/kg, i.p.) which is 3 to 30 times higher than those producing anxiolytic-like activity in the punished drinking (10 mg/kg, i.p.) and elevated plus-maze (1 mg/kg, i.p.) tests, while in the case of diazepam and lorazepam, this ratio was close to 1. We can therefore anticipate a higher therapeutic ratio of SL651498 compared with classical BZs in future clinical studies. On the sleep-wakefulness cycle in rats, SL651498 (10 mg/kg, p.o.) did not disrupt the sleep pattern and produced no alteration of paradoxical sleep. While the low intrinsic efficacy of SL651498 at the $GABA_A$ α_1 subtype may account for its limited propensity to produce sedation, the weak interaction of the compound with the α_5-containing $GABA_A$ receptor subtype may account for its profile in the grip strength and rotarod tests. (Turski et al., 1990; Griebel et al., 1999b). Consonant with this view is a recent finding that the preferential $GABA_A$ α_1 subtype antagonist β-carboline-3-carboxylate *t*-butyl ester did not block the myorelaxant effects of diazepam (Griebel et al., 1999a). Moreover, it was reported that mice carrying the H101R point mutation in the α_1 subunit still displayed muscle relaxation following the administration of diazepam (Rudolph et al., 1999).

In the present study, repeated treatment with diazepam produced tolerance to its anticonvulsant effects. In contrast, after repeated administration of SL651498, its anticonvulsant potency was not changed. Physical dependence was quantified in the present study by measuring the increase in the sensitivity to isoniazid upon discontinuation of repeated treatment at different times after drug withdrawal. In contrast to mice repeatedly treated with diazepam, those that received SL651498 did not show changes in their sensitivity to isoniazid following flumazenil challenge at any time postinjection, indicating a lack of precipitated-withdrawal signs. It is likely that the lack of occurrence of tolerance and physical dependence following chronic SL651498 may be due to its weak activity and/or partial agonist activity at the $GABA_A$ receptor α_1 and/or α_5 subtypes (O'Donovan et al., 1992). Indeed, a similar lack of tolerance to anticonvulsant action or increased sensitivity to seizures induced by convulsant challenge has been reported previously following chronic administration of ligands that have no affinity for the $GABA_A$ receptor α_5 subtype (e.g., zolpidem, zaleplon) or those that behave as partial agonists at this receptor (e.g., abecarnil, CL218,872) (VonVoigtlander and Lewis, 1991; Perrault et al., 1992, 1993; Sanger et al., 1996).

Some of the effects of ethanol are mediated by an action on

TABLE 8
Summary of the pharmacological properties of SL651498 in rodents
Comparison with diazepam and other BZs (unpublished data).

Tests	MED (*ED_{50}) i.p. (p.o.)			
	SL651498	Diazepam	Clorazepate	Lorazepam
		mg/kg		
Isoniazid-induced convulsions in mice	7*	0.7*	2*	2*
PTZ-induced convulsions in mice	9.5*	0.3*	0.4*	
Electroshock-induced convulsions in mice	≈40*	1.5*	2.5*	
Lever-pressing conflict test in rats	3	2.5	3	
Drinking conflict test in rats	10 (10)	1 (10)	10 (10)	0.1
Elevated plus-maze in rats	1 (3)	3 (20)	10 (10)	0.1 (1)
Light/dark test in mice	10	2	2	1
Four-plate test in mice	4	(2)		
Mouse defense test battery	3 (3)	0.5 (1)	10	
Rotarod in mice	100	10	3	1
Rotarod in rats	30	10	1	0.3
Grip Strength in mice	56*	3.5*		0.6*
Grip Strength in rats	27*	2.9*		0.3*
EEG in rats	30 (30)	1		0.1
Horizontal wire test in mice	(>30)	(1)		
Interaction with alcohol in mice	(30)	(1)		

MED, minimal effective dose; PTZ, pentylenetetrazole.

the $GABA_A$ receptor chloride channel complex, and BZs potentiate the depressant effects of ethanol (e.g., Lister and Linnoila, 1991). In line with these findings, results from the present study showed that diazepam potentiated the motor-impairing effects of ethanol in the horizontal wire test at all doses (from 1–10 mg/kg). SL651498 potentiated ethanol effects only at 30 mg/kg. While this effect appeared at a dose 10 times higher than the minimal effective anxiolytic dose, diazepam produced additive effects with ethanol at doses that overlapped with those eliciting anxiolytic-like activity.

In conclusion, the present study showed that the pyridoindole derivative, SL651498, is a functionally selective agonist at the $GABA_A$ receptor α_2 and α_3 subtypes and a partial agonist at α_1- and α_5-containing $GABA_A$ receptor subtypes. SL651498 displays clear anxiolytic-like activity in a variety of models in the absence of motor effects. The differential profile of SL651498 as compared with diazepam may be related to its different intrinsic efficacy and/or selectivity for certain $GABA_A$ receptor subtypes. SL651498 represents a promising alternative to agents currently used for the treatment of anxiety disorders without the major side effects seen with classical BZs.

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