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Stimulation of the β_3-Adrenoceptor as a Novel Treatment Strategy for Anxiety and Depressive Disorders

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The characterization of the first selective orally active and brain-penetrant β_3-adrenoceptor agonist, SR58611A (amibegron), has opened new possibilities for exploring the involvement of this receptor in stress-related disorders. By using a battery of tests measuring a wide range of anxiety-related behaviors in rodents, including the mouse defense test battery, the elevated plus-maze, social interaction, stress-induced hyperthermia, four-plate, and punished drinking tests, we demonstrated for the first time that the stimulation of the β_3 receptor by SR58611A resulted in robust anxiolytic-like effects, with minimal active doses ranging from 0.3 to 10 mg/kg p.o., depending on the procedure. These effects paralleled those obtained with the prototypical benzodiazepine anxiolytic diazepam or chlordiazepoxide. Moreover, when SR58611A was tested in acute or chronic models of depression in rodents, such as the forced-swimming and the chronic mild stress tests, it produced antidepressant-like effects, which were comparable in terms of the magnitude of the effects to those of the antidepressant fluoxetine or imipramine. Supporting these behavioral data, SR58611A modified spontaneous sleep parameters in a manner comparable to that observed with fluoxetine. Importantly, SR58611A was devoid of side effects related to cognition (as shown in the Morris water maze and object recognition tasks), motor activity (in the rotarod), alcohol interaction, or physical dependence. Antagonism studies using pharmacological tools targeting a variety of neurotransmitters involved in anxiety and depression and the use of mice lacking the β_3 adrenoceptor suggested that these effects of SR58611A are mediated by β_3 adrenoceptors. Taken as a whole, these findings indicate that the pharmacological stimulation of β_3 adrenoceptors may represent an innovative approach for the treatment of anxiety and depressive disorders.
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INTRODUCTION

Studies in humans and animals have shown a relationship between alterations in noradrenergic (NA) brain system function and behaviors associated with depression and anxiety (Ressler and Nemeroff, 2000; Millan, 2006). For example, the β_1 and β_2 adrenoceptors are known to participate in stress-related behavioral changes (Gorman and Dunn, 1993; Gurguis *et al*, 1999; Cecchi *et al*, 2002) and to mediate the effects of antidepressant treatment (Hancock and Marsh, 1985; Stahl *et al*, 1987; Manier *et al*, 1989; Holoubek *et al*, 2004). Among the β-adrenergic receptor subtypes, little is known about the central role of the G_S-protein-coupled β_3 subtype (Strosberg and Pietri-Rouxel, 1996), which, albeit mainly present in peripheral tissues, has been found in small quantities in the human and rat brain. Initially, the existence of the β_3 adrenoceptor in the brain was questioned, as classical binding studies had failed to detect its presence in central tissues. However, more recently, experiments using reverse transcription/PCR revealed the existence of β_3-adrenoceptor mRNA in discrete regions of the rat and human brain, including hippocampus, hypothalamus, amygdala, and cerebral cortex, areas known to be involved in the modulation of stress-related behaviors (Rodriguez *et al*, 1995; Summers *et al*, 1995; Strosberg, 1997). Furthermore, the availability of the selective β_3-adrenoceptor agonist SR58611A (amibegron) has opened new avenues for exploring the possible involvement of the β_3 adrenoceptor in the modulation of stress-related disorders (Bianchetti and Manara, 1990; Manara and Bianchetti, 1990). SR58611A, which is chemically related to the phenylaminotetralines family, displays high efficacy and potency at the rat and human β_3 receptor, 280- and 140-fold selectivity for rat β_3 as compared with β_1 and β_2 receptors, and is inactive at a number of other

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central targets involved in the regulation of stress-related disorders (Bianchetti and Manara, 1990; Simiand *et al*, 1992; Strosberg and Pietri-Rouxel, 1996). In a previous report, Simiand *et al* (1992) have shown that SR58611A behaved like an antidepressant in mechanistic models considered to be predictive of clinical antidepressant activity (ie, antagonism of drug-induced hypothermia, toxicity produced by yohimbine) and in the learned helplessness test in rats.

In the present series of experiments, the behavioral effects of SR58611A were examined using a variety of models covering various aspects of anxiety-related behaviors in rodents, including anticipatory anxiety in conflict procedures (punished drinking and four plate), neophobia in an exploration model (elevated plus-maze), social avoidance in social interaction paradigms, defensive aggression in a fear/anxiety defense test battery, and a procedure based on stress-induced changes in behavioral parameters (social defeat-induced anxiety). Moreover, the effects of SR58611A were investigated in acute (forced-swimming, tonic immobility) and chronic (chronic mild stress (CMS)) models used for the characterization of antidepressants. In this latter experiment, the compound was given repeatedly for about 5 weeks. In the light of numerous findings revealing the ability of antidepressant drugs to interfere with sleep architecture (Sharpley and Cowen, 1995), effects of SR58611A on spontaneous sleep parameters in rat were also evaluated. Comparative data for the anxiolytic diazepam and the antidepressants imipramine and fluoxetine, obtained under the same experimental conditions, are also provided. In addition, possible unwanted effects related to cognition, motor activity, alcohol interaction, or physical dependence of increasing doses of SR58611A were examined.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley or Wistar rats (Iffa Credo, L'Arbresle, and Charles River, Saint-Aubin-lès-Elbeuf, France), housed in groups of two to eight and weighing 180–370 g at the time of testing, were used. Male Long Evans rats (400–500 g, Iffa Credo) were used in the mouse defense test battery (MDTB). Male Mongolian gerbils (CERJ, Le Genest St-Isle, France) housed in groups of five to six and weighing 45–70 g at the time of testing, were used. Male CD1 (10-week-old, rotarod), NMRI (6-week-old, social defeat stress-induced anxiety), OF1 (10-week-old, MDTB), C57Bl/6j (7-week-old, social interaction), and BALB/c (6-week-old, CMS procedure) mice, weighing 17–32 g at the time of testing were supplied by Iffa Credo or CERJ. The choice of the strains was made on the basis of previous (published and unpublished) studies indicating that level of responsiveness (with or without treatment) in a given test may dramatically differ between strains. For example, BALB/c mice are particularly indicated when using CMS test as they generally display very constant baseline performance (in contrast to other strains, especially those from outbred lines). For studies with the β_3-adrenoceptor null mice, we used 10-week-old male FVB/NJ and aged-matched FVB/NJ male littermate mice with a targeted disruption of the β_3 adrenoceptor bred in Sanofi-Aventis care unit. They were generated as described by Revelli *et al* (1997). Preliminary experiments indicated that both β_3-adrenoceptor null mice and their wild-type (WT) counterparts were not suitable for behavioral experiments, showing for example minimal defensive responses in the MDTB. All mice were grouped in cages of 10, except for MDTB, predator odor stress-induced hyperthermia, and CMS tests where they were housed singly. All animals were maintained under standard laboratory conditions (21–24°C) and kept on a 12 h light/dark cycle with light on at 0700. All procedures have been approved by the Animal Care and Use Committee of Sanofi-Aventis Research and fully comply with French legislation (decree 87-848, October 19, 1987; and order from April 19, 1988), which implemented the European directive (86/609/EEC) on research involving laboratory animals.

Drugs

SR58611A, diazepam, chlordiazepoxide, flumazenil, and fluoxetine were synthesized by the Medicinal Chemistry Department of Sanofi-Aventis except imipramine, apomorphine, *p*-CA (*p*-chloroamphetamine), ICI-118,551, betaxolol, and propranolol supplied by Sigma-Aldrich (St Quentin Fallavier, France). Compounds were prepared as solutions or suspensions in physiological saline or distilled water containing Tween 80. Drugs were given intraperitoneally (i.p.), subcutaneously (s.c.), or per os (p.o.) in a volume of 5 (rats) or 20 ml/kg (mice and gerbils).

Models of Anxiety

The elevated plus-maze test in rats. The procedure is based on that described by Pellow *et al* (1985). The testing apparatus was made of polyvinylchloride (PVC) and elevated to a height of 70 cm with two open/unprotected (50×10 cm) and two enclosed arms ($50 \times 10 \times 50$ cm) arranged so that the arms of the same type were opposite to each other. The apparatus used for rats was equipped with infrared beams and sensors capable of measuring activity in the different arms. The light intensity measured in the open arms was 10 lux. For testing, rats were placed in the center of the maze facing a closed arm, for a free exploration period of 4 min. 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 the number of entries into open arms to total number of entries in both open and closed arms, and mean number of entries into closed arms. Compounds were administered p.o. 60 min before testing.

The social defeat stress-induced anxiety in the elevated plus-maze in mice. The procedure was a modification of the technique described by Miczek (1979). A naïve mouse was placed in the cage of a resident male aggressor, which was selected for high levels of aggression. The intruder was removed from the area when it displayed a submissive posture after being attacked and placed in a wire mesh enclosure to avoid physical contact or injury. Then it was returned to the resident aggressor cage for 60 min. At the end of the interaction period, the intruder mouse was placed onto the central platform of the elevated plus-maze during a 5-min period. Results were expressed as mean ratio of time spent in open arms to total time spent in both open

and closed arms. The compounds were administered p.o. 30 min before social defeat.

The mouse defense test battery. The test was conducted in an oval runway as described by Griebel *et al* (1997). Mice were placed in the runway for an initial 3-min locomotor activity testing during which line crossings were recorded. Immediately after this period, the experimenter introduced a hand-held dead rat at one extremity of the runway and brought it up to the subject for five times. If the subject fled, a rat avoidance was recorded. Then, the rat was again brought up to the subject and the number of stops and chase speed were recorded. By closing two doors, the runway was converted into a straight alley. The rat was introduced at one extremity of the alley and the number of attempts at approaching the rat (approaches/withdrawals) was recorded during 30 s. Finally, the experimenter brought the rat up to contact the subject. Upright postures and bites were noted. Drugs were administered p.o. 60 min before testing.

Predator odor stress-induced hyperthermia in β_3 adrenoceptor null and wild-type mice. The stress procedure was adapted from that originally described by Zethof *et al* (1995). A singly housed mouse was placed for 10 min in a plastic cage ($35 \times 35 \times 20$ cm) containing freshly soiled bedding from a rat cage. Body temperature was measured at three occasions, that is, before p.o. drug treatment ($T0$), 50 min after drug treatment and before predator odor stress exposure ($T50$), and 60 min after drug treatment and immediately after predator odor stress exposure ($T60$). The effects of the compounds on basal temperature levels were evident at $T50$, while $T60-T0$ was considered to reflect stress-induced hyperthermia.

The four-plate test in gerbils. The test apparatus is based on the one described by Boissier *et al* (1968) for mice and adapted to gerbils. The apparatus consists of a cage with a floor composed of four square metal plates connected to a device that can generate electric shocks (1.5 mA; 0.3 s). After a 15-s latency period, the animal is subjected to an electric shock every time it moves from one plate to another. The number of punished crossings is recorded during a 3-min testing period. Experiments were carried out 60 min after acute or chronic (8 days) oral administration of SR58611A.

The punished drinking test in rats. The procedure was a modification of that described by Vogel *et al* (1971). Rats were deprived of water for 48 h before testing and were placed in cages with a stainless steel grid floor (MED Associates Inc., St Albans, VT). Each cage contained a drinking tube connected to a 50-ml burette filled with tap water. Trials were started when the rat's tongue came in contact with the drinking tube for the first time. An electric shock (0.5 mA, 600 ms) was delivered to the tongue every 20 licks. The number of shocks (punished responses) was recorded during a 5-min period. SR58611A was administered i.p. either alone or in combination with the benzodiazepine (BZ) antagonist flumazenil, the nonselective β_1/β_2 antagonist propanolol, the selective β_1-antagonist betaxolol, the selective β_2-antagonist ICI-118,551, or the 5-HT depletor *p*-CA (the last drug was given 8 days before testing).

The social interaction test in gerbils and mice. This test was initially described for gerbils by File *et al* (2001). Male gerbils were habituated individually to freely explore an experimental plastic box ($30 \times 30 \times 20$ cm) under bright light (300 lux) during 10 min. The following day, two gerbils from the same weight but from different cages received the same treatment and were subsequently placed in single cages. Then, they were placed together in the experimental box for a 4 min 30 s observation period. During this period, social interaction duration (in seconds) was recorded manually and included of active behaviors such as grooming, chasing, and playing. Simultaneously, locomotor activity (in cm) for each gerbil was measured by an automated tracking system (Ethovision, Noldus, The Netherlands). This testing procedure was adapted to male mice (same weight, same treatment for each pair, mice originating from different cages). In mice, social interactions included in brief contacts between the nose of one mouse and the nose, the head, or the flank of its partner. No habituation session was provided to mice and social interactions were measured during a 5-min testing session under moderate lightning (100 lux) conditions. Locomotor activity was also automatically recorded for each mouse by the Ethovision system. Drugs were administered p.o. 60 min before testing in gerbils and mice. In a complementary experiment, measure of social interaction in mice using the same procedure was used to assess the effect of abrupt discontinuation of treatments on anxiety levels (withdrawal effect). For this purpose, daily i.p. injections of SR58611A and diazepam were performed during 10 days followed by a 72 h washout period. Acute control treatments were given i.p. 30 min before testing in mice that received control injections during 10 days.

Models of Depression

The forced-swimming test in rats. The procedure was a modification of that described by Porsolt *et al* (1977). Rats were placed in individual glass cylinder (40 cm height, 17 cm diameter) containing water (21°C) to a height of 30 cm. Two swimming sessions were conducted (an initial 15-min pretest followed 24 h later by a 6-min test). The duration of immobility was measured during the 6-min test. Drugs were given p.o. twice (15 min after the first session on day 1 and 60 min before the test on day 2).

The pinch-induced tonic immobility paradigm in gerbils. The test is based on that described by Salomé *et al* (2006). To induce tonic immobility, animals (6–11 per group) were held on a flat surface and were firmly pinched for 15 s at the scruff of the neck and the back. They were then placed on parallel bars (4 mm in diameter, 28 cm long, spaced 5 cm apart and having a 3 cm difference in height). The front paws were placed gently on the upper bar (situated at 43 cm above the base) and the hind paws on the lower bar. The duration of tonic immobility was measured in five successive trials with a 30 s inter-trial interval. Each trial ended when an animal started to move, or after 90 s of immobility. Preliminary studies showed that after five

successive trials, 98% of untreated gerbils presented tonic immobility. Experiments were performed 60 min after p.o. or 30 min after i.p. administration of the drugs.

Apomorphine-induced hypothermia in β_3-adrenoceptor null and wild-type mice. Mice were placed in individual cages ($10 \times 10 \times 15$ cm) for 60 min and their rectal temperature was recorded. Apomorphine (16 mg/kg, s.c.) was given 30 min after SR58611A (1 mg/kg, i.p.), imipramine (10 mg/kg, i.p.), or vehicle. Rectal temperature was measured again 30 min after the apomorphine treatment.

The CMS procedure in mice. The CMS protocol is based on a protocol originally designed by Willner *et al* (1992) for rats and by Griebel *et al* (2002b) for mice. The protocol consists of the sequential and unpredictable application of a variety of mild stressors (for example, restraint, forced-swimming, water and/or food deprivation) during 6 weeks (Griebel *et al*, 2002a, b). Administration of SR58611A (3 mg/kg) or fluoxetine (10 mg/kg) was started 2 weeks after the beginning of CMS. Animals (21–28 g at the start of the experiment) were treated p.o. once a day until all experiments were completed (33 days). The consequence of the decrease in grooming behavior seen in stressed animals was in the progressive degradation of the physical state of the coat (loss of fur and dirty fur), which can be measured using the following scale: 3 points: clean and well-groomed coat; 2 points: disorganized (poorly groomed) coat on the back; 1 point: dirty coat with loss of patches of fur. Based on these observations, we measured physical state once a week over the entire CMS period. At the end of the CMS procedure, mice were tested in elevated plus-maze under moderate lightning conditions (30 lux in open arms) to assess the impact of CMS on anxiety levels.

ECoG and sleep/waking cycle in rats. Cortical electrodes were placed on the sensorimotor cortex of rats in order to allow reliable visual discrimination of wakefulness (W; low voltage electrocortical activity), slow-wave sleep (SWS; increase in electrocortical activity) and rapid eye movement or paradoxical sleep (REM; hypersynchronization of the theta rhythm in the visual area). Sleep/waking parameters were recorded between 1100 and 1700 on a control day (i.p. injection of vehicle) and on a subsequent drug day (i.p. injection of SR58611A or fluoxetine). Analysis of the signal was performed by a computerized system discriminating between the various sleep phases using sequential spectral analysis of 10-s periods (Deltamed's software 'Coherence').

Evaluation of Potential Side-Effects of SR58611a

Motor coordination in mice and interaction with ethanol in the rotarod test. Mice were tested in an apparatus equipped with a rotating bar (speed: 5 turns/min, diameter: 1 cm; length 5 cm). Sixty minutes after oral administration of SR58611A diluted either in water or in a 20% ethanol solution, mice were placed on the turning rotarod. The experiment lasted 2 min. The latency to fall from the bar (in seconds) was measured manually.

Spatial reference-memory in the rat Morris water maze test. The Morris water maze apparatus consisted of a circular arena (150 cm diameter, 60 cm high) made of gray PVC and filled with water (temperature $= 28 \pm 2^{\circ}$C) to a height of 30 cm and made opaque with addition of milk (Morris, 1984). A square Plexiglas platform (12 cm^2) was submerged 1 cm underneath the water and made invisible for the rat. Testing lasted for 3 days and each rat was submitted to three consecutive trials per day. The platform position remained the same for all trials but starting positions changed from one trial to the other. The animal was released in the water and a maximum of 120 s was given to reach the platform. When the rat had climbed onto the platform, it was left over there for 30 s before the next trial was started. If the rat did not find the platform on time, it was placed on it by the experimenter and left for 30 s. Latencies (in seconds) to reach the platform were recorded and were expressed as their means over the 3 consecutive days of testing. SR58611A and diazepam were administered p.o. or i.p., 60 or 30 min, respectively, before each testing session.

Short-term episodic memory in the rat object recognition task. Rats were tested on a visual recognition memory test similar to that described by Ennaceur and Delacour (1988). The apparatus consisted of uniformly lit hardboard enclosure ($65 \times 45 \times 45$ cm) observed via a video monitoring system. Each experiment consisted of three sessions. The first session consisted of a 2-min context habituation session. The animals were again placed in the enclosure 1 h later for a second session (learning session), in the presence of two identical objects. The amount of time necessary to explore the two objects during a total of 20 s was recorded manually. Cutoff time was set at 5 min. Exploration was defined as rat having its head within 2 cm of the object while looking at, sniffing or touching it. The third session (recall session) took place 1 h later. A novel object was substituted from one of the previously presented. Time spent exploring the familiar and novel objects was recorded over 3 min. Drugs were administered p.o. twice, 60 min before the habituation session and 60 min before the learning session.

Sequential spectral analysis of ECoG in rats. Rats were anesthetized with sodium pentobarbital (Virbac, Carros France, 50–60 mg/kg i.p.) and mounted in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA, USA). Cortical electrodes (small stainless steel screw electrodes 0.9 mm in diameter) were screwed into the bone over the sensorimotor cortex (1.5 mm lateral to the median suture and 1.5 mm behind the fronto-parietal 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). Cortical electrodes were attached to a connector (Winchester, 7-lead) and fixed with dental cement to the cranium. After a postoperative recovery period of 2–3 weeks, single-housed animals were placed in plexiglas cylinders with free access to food and water. The sequential spectral analysis was assessed with ECoG recording during 1 h after sleep was started and compared the effects of SR58611A and diazepam. The analysis distinguished six frequency bands in the rat: 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). Analysis of the signal was performed automatically as described previously.

Statistical analyses. Data from anxiety and depression models were either analyzed by parametric one-way analysis of variance (ANOVA) followed by Dunnett or Newman–Keuls test, or nonparametric χ^2 test followed by Kruskal–Wallis multiple comparisons tests. Water maze and object recognition data were analyzed by two-way ANOVAs (treatment × day, treatment × object) with repeated measures as well as apomorphine induced hypothermia data (treatment × genotype) followed by Newman–Keuls or Dunnett tests. EEG spectral analysis parameters and sleep stages were analyzed either with a Student's test or a repeated measure ANOVA (treatment × day). Statistical significance was set at 0.05.

RESULTS

Models of Anxiety

The elevated plus-maze test in rats. SR58611A (from 0.3 mg/kg) and diazepam (at 3 mg/kg) significantly increased exploration of the open arms of the elevated plus-maze in rats ($\chi^2 = 19.9$, $P < 0.001$, Figure 1a) and the percentage of entries into the open arms ($\chi^2 = 21.7$, $P < 0.001$, Figure 1b). The number of entries into closed arms, a presumed index of locomotion, was not modified by the treatment (Figure 1c).

The social defeat stress-induced anxiety in the elevated plus-maze in mice. Social defeat stress in mice significantly decreased open-arm exploration of the elevated plus-maze ($P < 0.01$, Figure 2). This effect was blocked by SR58611A ($F_{4,44} = 4.31$, $P < 0.01$) at 10 mg/kg, and diazepam produced similar effects ($F_{3,36} = 14.95$, $P < 0.001$) at 1 mg/kg.

The mouse defense test battery. The results are presented in Table 1. Before exposure to the rat, neither SR58611A nor diazepam modified significantly the number of line crossings. In the rat avoidance test, SR58611A and diazepam significantly decreased avoidance frequency (SR58611A: $\chi^2 = 9.0$, $P < 0.05$; diazepam: $\chi^2 = 9.1$, $P < 0.05$) at 3 mg/kg. When mice were chased by the rat, escape speed was not modified by either treatment. However, SR58611A reduced significantly the number of stops ($\chi^2 = 21.8$, $P < 0.001$) at 1 and 3 mg/kg. Diazepam produced similar effects ($\chi^2 = 22.4$, $P < 0.001$) at 1 mg/kg. Moreover, at 3 mg/kg, both SR58611A and diazepam increased significantly the number of approaches of the rat followed by withdrawal ($\chi^2 = 13.1$, $P < 0.01$ and $\chi^2 = 14.0$, $P < 0.01$, respectively). When the mouse was directly confronted with the rat, SR58611A significantly decreased upright postures and bitings ($\chi^2 = 14.5$, $P < 0.01$ and $\chi^2 = 17.1$, $P < 0.001$, respectively) at 1 and 3 mg/kg (Figure 3). Diazepam produced similar effects at 3 mg/kg (postures $\chi^2 = 16.2$, $P < 0.01$; bitings $\chi^2 = 11.7$, $P < 0.01$).

Predator odor stress-induced hyperthermia in β_3-adrenoceptor null and wild-type mice. Because the FVB genetic background of β_3-adrenoceptor null (ADRB3 knockout (KO)) mice was not suitable for models involving a behavioral observation, effects of SR58611A were evaluated on an autonomic response in a predator odor stress-induced hyperthermia test in WT and ADRB3 (KO) mice.

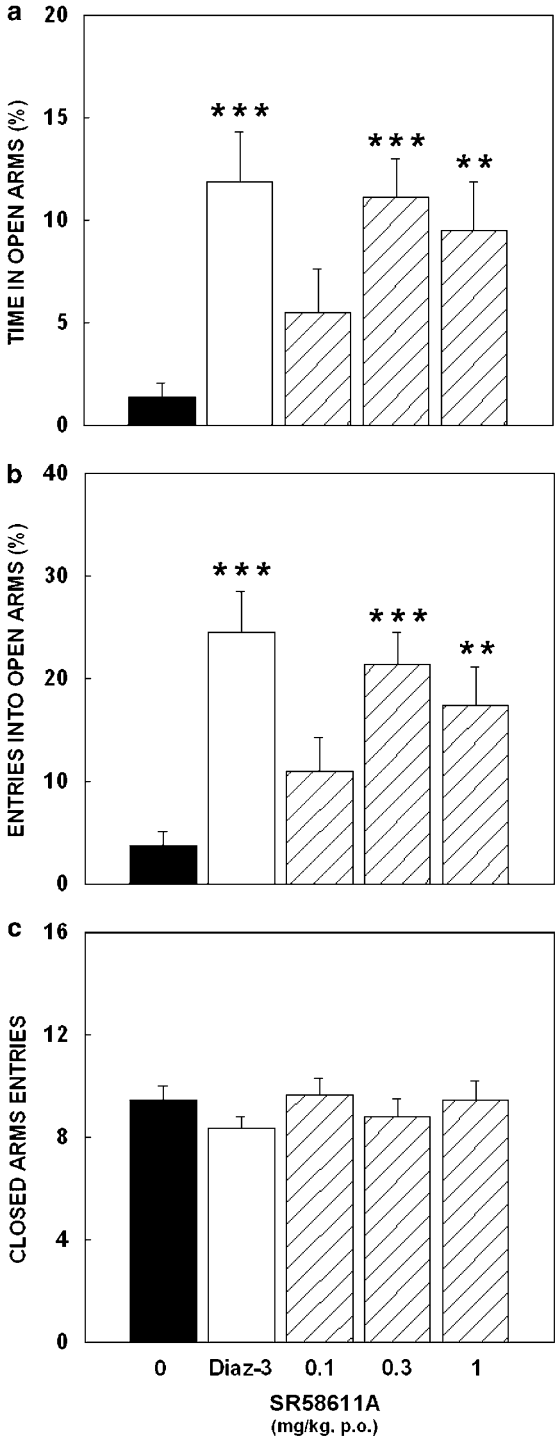

Figure 1 Effects of SR58611A and diazepam in the elevated plus-maze test. Drugs were administered orally 60 min before testing. Data represent (a) mean percentage of time spent in the open arms + SEM, (b) mean percentage of entries in the open arms + SEM, and (c) mean number of entries into closed arms + SEM, $^{**}P < 0.01$, $^{***}P < 0.001$, $N = 13$–14.

There was no genotype effect on basal rectal temperature at *T*0 (not shown). SR58611A (3 and 10 mg/kg) potently reduced hyperthermia in WT mice ($F_{3,89} = 4.9$, $P < 0.01$, SR58611A 3 mg/kg *vs* control: $P < 0.01$, SR58611A 10 mg/kg *vs* control: $P < 0.001$), but failed to produce the same effect in ADRB3 (KO) mice (Figure 4). In contrast, chlordia-

zepoxide (10 mg/kg) blocked hyperthermia in both WT ($P<0.01$) and ADRB3 (KO) mice ($P<0.05$). SR58611A and chlordiazepoxide had no effect on basal rectal temperature at *T*50 (not shown).

The four-plate test in gerbils. SR58611A significantly increased punished crossings in the four-plate test in gerbils after acute and repeated (8 days) treatments at 3 and 10 mg/kg. Diazepam produced similar effects after an acute treatment at 1 and 2 mg/kg (Figure 5a and b, $F=3.69$, $P<0.05$ and $F=13.1$, $P<0.001$, respectively).

The punished drinking test in rats. SR58611A increased significantly punished responses at 3 and 10 mg/kg ($\chi^2=11.9$, $P<0.05$), an effect shared by diazepam at 3 mg/kg (Figure 6). Moreover, the effects of SR58611A were not altered by flumazenil (10 mg/kg), propranolol (8 mg/kg), betaxolol (5 mg/kg), ICI-118,551 (5 mg/kg), or *p*-chloroamphetamine (2×10 mg/kg). Morphine did not modify punished responding at doses ranging from 0.3 to 3 mg/kg (Table 2).

The social interaction test in gerbils and mice. SR58611A significantly increased social interaction in gerbils ($F=20.3$, $P<0.001$) and mice ($\chi^2=29.6$, $P<0.001$) at 1 mg/kg p.o, without affecting locomotion (not shown). Diazepam induced similar increases at 0.1 mg/kg in gerbils and at 1.5 mg/kg p.o. in mice. In mice, using high doses of SR58611A (10 mg/kg, i.p.) and diazepam (5 mg/kg, i.p.) administered during 10 days and followed by an abrupt discontinuation (72 h) of the treatment, social interactions were significantly modified ($\chi^2=20.2$, $P<0.001$). A significant increase in social interaction was observed in mice withdrawn from SR58611A ($P<0.05$, +52%). On the contrary, a tendency for a decrease in the duration of interaction was observed in mice withdrawn from diazepam (−25%). Single administration of either treatment in mice

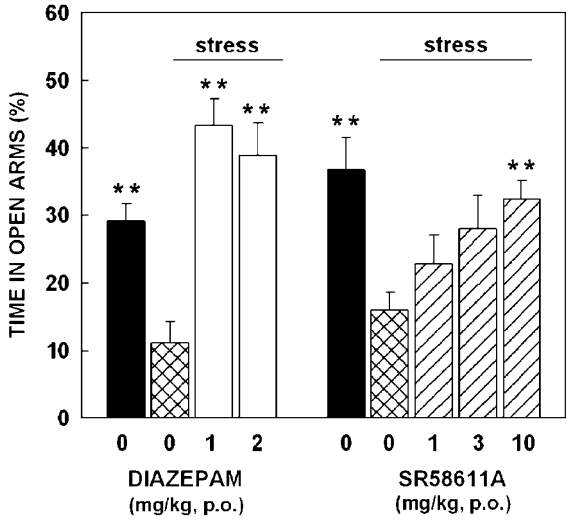

Figure 2 Effects of SR58611A and diazepam on social defeat stress-induced anxiety in the elevated plus-maze in mice. The compounds were administered p.o. 30 min before social defeat and 60 min before exposure to the elevated plus-maze. Data represent mean percentage of time spent in the open arms + SEM, $^{**}P<0.01$ (Dunnett).

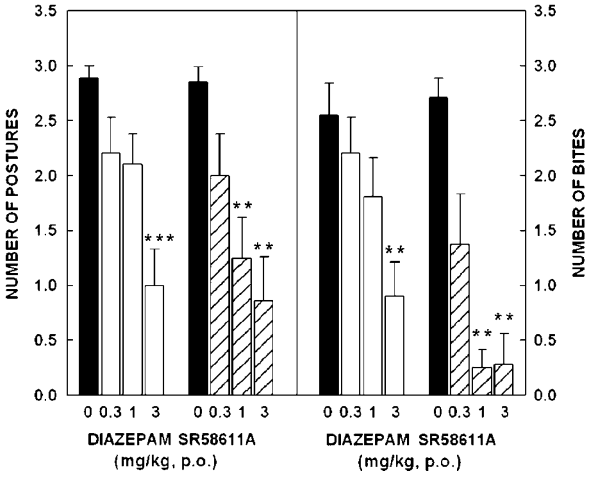

Figure 3 Effects of SR58611A and diazepam on defensive aggression (upright postures and bitings) in the MDTB. Drugs were administered p.o. 60 min before testing. Data represent mean + SEM, $^{**}P<0.01$, $^{***}P<0.001$, $N=7$–11.

Table 1 Effects of SR58611A in the Mouse Defense Test Battery

		Activity		Flight	Risk assessment	
Compound	**Dose (mg/kg p.o.)**	**Line crossings**	**Chase speed (m/s)**	**avoidance frequency**	**Stops**	**Approaches withdrawals**
SR58611A	0	119.57±6.32	0.72±0.05	3.85±0.34	9.28±0.28	0.00±0.00
	0.3	124.13±7.08	0.59±0.06	2.63±0.26	8.12±0.23	0.00±0.00
	1	110.63±6.43	0.61±0.06	2.75±0.37	6.62±0.42**	0.37±0.26
	3	108.43±7.56	0.60±0.07	2.29±0.29*	5.71±0.36***	1.28±0.47*
Diazepam	0	111.78±7.07	0.66±0.20	3.44±0.34	7.55±0.53	0.11±0.11
	0.3	112.90±7.72	0.54±0.14	2.20±0.42	6.50±0.72	0.10±0.10
	1	134.73±8.49	0.53±0.06	2.64±0.45	4.18±0.72*	0.63±0.20
	3	104.80±8.63	0.46±0.15	1.30±0.50*	1.60±0.56***	1.70±0.45**

Data represent mean±SEM.
$^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, multiple comparison test, *vs* control, $N=7$–11.

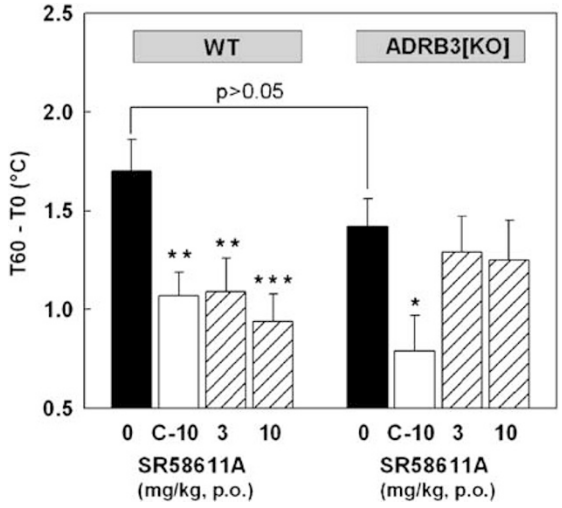

Figure 4 Effect of SR58611A and chlordiazepoxide (C-10 = 10 mg/kg, p.o.) in ADRB3 KO and WT mice on predator odor stress-induced hyperthermia. Body temperature was measured at three occasions, that is, before p.o. drug treatment (*T*0), 50 min after drug treatment and before predator odor stress exposure (*T*50), and 60 min after drug treatment and immediately after predator odor stress exposure (*T*60). Data represent mean ± SEM difference in rectal temperature between *T*0 and *T*60, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $N=13$–21.

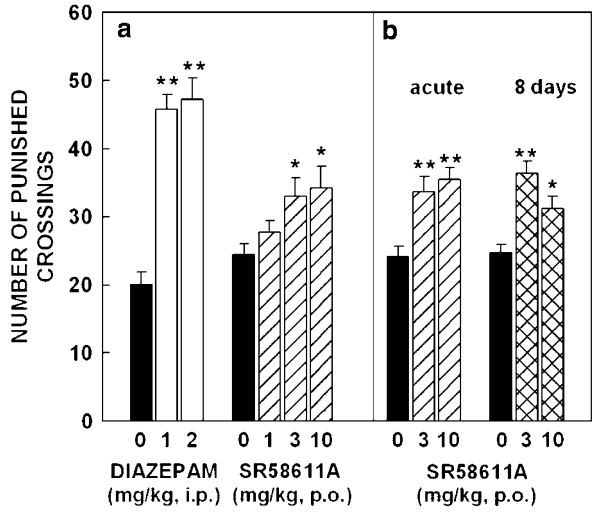

Figure 5 Effects of acute (a) and repeated (b) oral treatment with SR58611A in the four-plate test in gerbils. Experiments were carried out 60 min after acute or chronic (8 days) oral administration of SR58611A. In (b), acute indicates that animals were treated repeatedly with vehicle and administered with SR58611 the day of testing. Data represent mean + SEM, $^{*}P<0.05$, $^{**}P<0.01$, *vs* respective control group, $N=12$.

that received a control injection during 10 days produced significant interaction increases (acute diazepam *vs* controls $P<0.05$, +74%; acute SR58611A *vs* controls, $P<0.05$, +49%) (Table 3).

Models of Depression

The forced-swimming test in rats. The duration of immobility measured in the forced-swimming test in rats

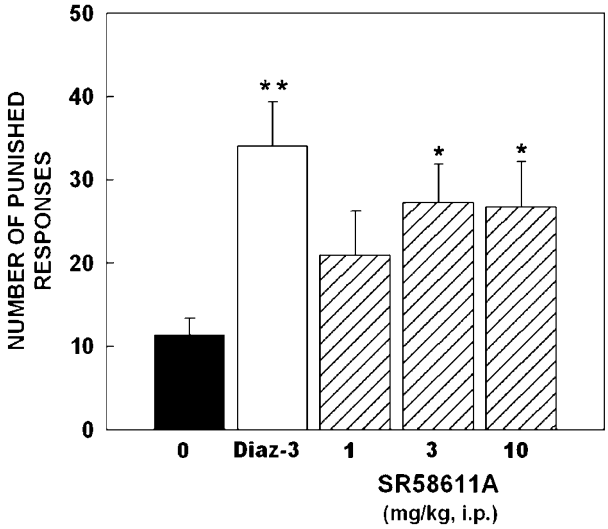

Figure 6 Effects of SR58611A and diazepam in the punished drinking test in rats. Drugs were administered i.p. 30 min before testing. Data represent mean + SEM, $^{*}P<0.05$, $^{**}P<0.01$, multiple comparison test *vs* control, $N=12$.

was significantly reduced by SR58611A ($F=4.19$, $P<0.05$, Figure 7) at 1 mg/kg and by fluoxetine ($F=6.0$, $P<0.01$) at 3 mg/kg.

The pinch-induced tonic immobility paradigm in gerbils. The duration of tonic immobility in gerbils was significantly decreased by SR58611A ($F=16.4$, $P<0.001$) at 3 mg/kg. A decrease of the same amplitude was observed with imipramine ($F=34.2$, $P<0.001$) at 7.5 mg/kg i.p. (Figure 8).

Apomorphine-induced hypothermia in β_3 adrenoceptor null and wild-type mice. SR58611A antagonized hypothermia induced by apomorphine in WT mice, but failed to produce the same effect in ADRB3 (KO) mice (treatment effect: $F=16.6$, $P<0.001$; genotype effect: $F=35.9$, $P<0.001$). In contrast, imipramine antagonized hypothermia induced by apomorphine in both WT and ADRB3 (KO) mice (treatment effect: $F=67.8$, $P<0.001$) (Figure 9).

The chronic mild stress procedure in mice. Over the 6 weeks of chronic stress regimen, there was a significant and progressive degradation of the physical state of the mice measured by an alteration in the general aspect of the coat (Figure 10a). This effect reached statistical significance from the second week ($P<0.01$). The physical degradation was significantly improved ($\chi^2=7.5$, $P<0.05$) by SR58611A from the second week of treatment (4th week of stress) ($P<0.05$), an effect which was of the same amplitude as that observed with fluoxetine ($P<0.05$) and which lasted until the CMS was over. Moreover, SR58611A, but not fluoxetine, fully reversed the anxiogenic-like effect of chronic stress ($\chi^2=9.2$, $P<0.01$, stressed controls *vs* stressed SR58611A: $P<0.05$, Figure 10b) in the elevated plus-maze test. It is noteworthy that neither fluoxetine nor SR58611A modified body weight in stressed animals throughout the experiment.

ECoG and sleep/wakefulness cycle in rats. SR58611A at 3 mg/kg did not significantly disrupt the pattern of the

Table 2 Effects of Flumazenil, Propranolol, ICI-118,551, Betaxolol, and *p*-CA on SR58611A-Induced Increase in Punished Responses and Effects of Morphine in the Punished Drinking Test in Rats

Compound	**Control**	**Diazepam**	**SR58611A**	**Flumazenil**	**SR58611A+Flumazenil**
Dose in mg/kg, i.p.		—	10	10	10+10
Punished responses	12.8±1.5	NT	21.8±2.8*	15.2±2.2	20.9±2.7*
% variation			+70*	+19	+63*
Compound	**Control**	**Diazepam**	**SR58611A**	**Propranolol**	**SR58611A+propranolol**
Dose in mg/kg, i.p.		3	10	8	10+8
Punished responses	8.3± 1.3	36.9±4.2***	27.5±4.4***	18.4±3.5	35.2±3.9***
% variation		+344***	+232***	+122	+324***
Compound	**Control**	**Diazepam**	**SR58611A**	**ICI-118,551**	**SR58611A+ICI-118,551**
Dose in mg/kg, i.p.		3	10	8	10+8
Punished responses	12.7±1.8	32.6±4.0***	26.8±3.9*	21.1±4.4	26.1±3.5*
% variation		+157***	+111*	+66	+106*
Compound	**Control**	**Diazepam**	**SR58611A**	**Betaxolol**	**SR58611A+Betaxolol**
Dose in mg/kg, i.p.		3	10	8	10+8
Punished responses	9.1±1.7	34.3±4.5***	25.3±4.5*	14.0±3.6	24.3±6.0*
% variation		+277***	+177*	+53	+167*
Compound	**Control**	**Diazepam**	**SR58611A**	***p*-CA**	**SR58611A+*p*-CA**
Dose in mg/kg, i.p.		3	10	8	10+8
Punished responses	16.9±3.3	31.7±3.8*	30.7±4.8*	20.3±4.5	35.4±4.0**
% variation		+88*	+82*	+20	+110**
Compound	**Control**	**Diazepam**	**Morphine**	**Morphine**	**Morphine**
Dose in mg/kg, i.p.		3	0.3	1	3
Punished responses	16.7±3.8	29.8±4.9*	12.7±3.8	13.7±3.3	14.1±3.2
% variation		+78*	−24	−18	−16

Data represent mean±SEM.
*$P<0.05$; **$P<0.01$; ***$P<0.001$; $N=15$–37.

sleep/wake cycle in rats, nor did it enhance SWS or alter REM sleep (Table 4). However, at the dose of 10 mg/kg, SR58611A significantly reduced REM sleep (−45%, $P<0.01$) and increased delay of REM onset (+392%, $P<0.01$) without altering W or SWS duration. Comparable sleep patterns were found in rats treated with fluoxetine at 10 mg/kg (decrease in REM sleep: −73%, $P<0.05$; increase in delay of REM onset: +198%, $P<0.01$).

Evaluation of Potential Side-Effects of SR58611A

Motor coordination in mice and interaction with ethanol in the rotarod test. SR58611A had no effect on the time spent on the rod (all mice stood on the rod for the 2-min test), while diazepam significantly decreased the latency to fall ($F=16.19$, $P<0.001$) at 10 mg/kg. A subactive (3 mg/kg) dose of diazepam, coadministered with a 20% ethanol solution, produced a significant decrease in latency to fall ($F=4.2$, $P<0.05$), while ethanol administration had no effect in SR58611-treated mice (Table 5).

Spatial reference memory in the Morris water maze test and short-term episodic memory in the object recognition task in rats. Latencies to reach the hidden platform significantly improved over the 3 days of testing in control animals ($F=10.35$, $P=0.0001$) and in SR58611A-treated rats at 1 mg/kg ($F=12.66$, $P<0.0001$), 3 mg/kg ($F=5.30$, $P<0.01$), and 10 mg/kg ($F=10.26$, $P<0.001$) (Table 6). There was no significant treatment effect ($F=1.134$, $P>0.05$), suggesting unaltered spatial reference memory performance in the presence of SR58611A at any dose tested. In contrast, animals treated with the two highest doses of diazepam showed no improvement in performance with repetition of acquisition sessions compared to controls rats ($F=5.74$, $P<0.01$), suggesting a significant deficit of spatial reference memory acquisition. In the object recognition task, SR58611A did not modify significantly locomotor activity during the habituation session or total time used to explore the objects for 20 s during the learning session (Table 6). During the recall session, after a short (1 h) delay, control rats and rats treated with SR58611A spent significantly more time exploring the novel object

Table 3 Effects of SR58611A and Diazepam in the Social Interaction Test: Dose Responses in Gerbils and Mice and Withdrawal Effect in Mice

Gerbils			Mice		
Compound	**Dose**	**Duration of social interactions (s)**	**Compound**	**Dose**	**Duration of social interactions (s)**
	mg/kg, p.o. (acute)			mg/kg, p.o. (acute)	
SR58611A	0	35.4 ± 2.3	SR58611A	0	11.0 ± 1.1
	1	55.8 ± 4.1***		0.3	15.1 ± 0.9
	3	64.2 ± 2.7***		1	17.0 ± 1.4*
	10	71.0 ± 4.1***		3	23.5 ± 3.8***
Diazepam	0	35.2 ± 3.4	Diazepam	1.5	24.7 ± 2.8***
	0.1	50.8 ± 2.6**		mg/kg, i.p. during 10 days (+72 h withdrawal)	
	0.3	54.2 ± 2.0***	SR58611A	0	14.2 ± 1.5
	1	56.0 ± 3.4***		10	21.6 ± 1.7*
				10 (acute)	21.2 ± 0.9*
			Diazepam	5	10.7 ± 2.6
				5 (acute)	24.7 ± 3.3*

Data represent mean ± SEM.
*$P<0.05$, **$P<0.01$, ***$P<0.001$, multiple comparison test or Dunnett *vs* control, $N=5$ pairs of gerbils and $N=9$–15 pairs of mice per group.

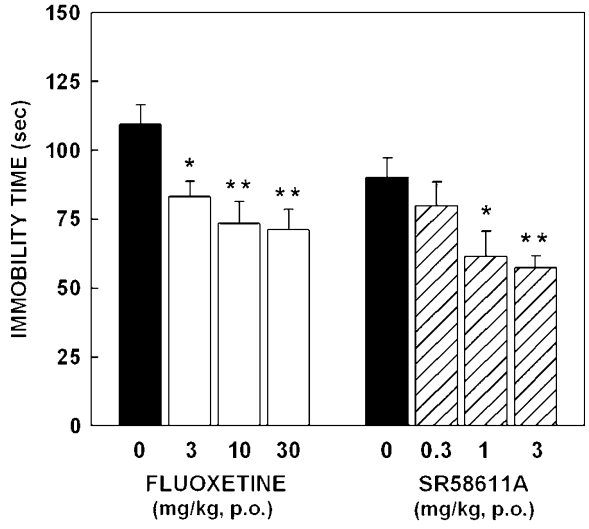

Figure 7 Effects of SR58611A and fluoxetine in the forced-swimming test in rats. Drugs were given p.o. twice (15 min after the first session on day 1 and 60 min before the test on day 2). Data represent mean + SEM, *$P<0.05$, **$P<0.01$, $N=7$.

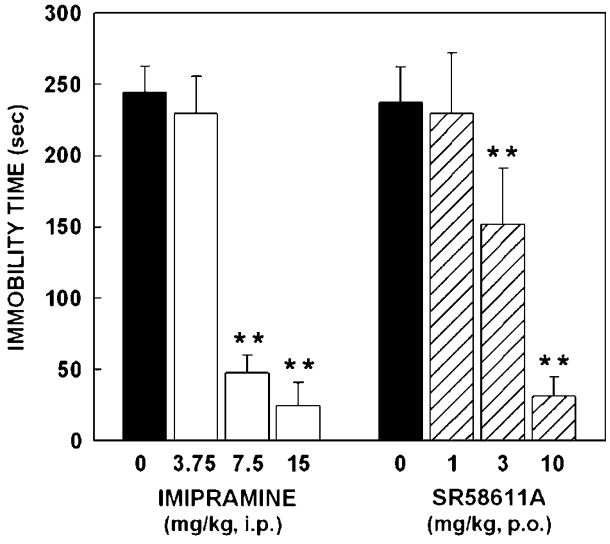

Figure 8 Effects of SR58611A and imipramine on pinch-induced tonic immobility in gerbils. Experiments were performed 60 min after p.o. or 30 min after i.p. administration of the drugs. Data represent mean + SEM, **$P<0.01$, $N=7$–8.

($F=180.46$, $P<0.0001$), suggesting preserved short-term episodic memory. In contrast, diazepam reduced significantly exploration of the novel object at 1 mg/kg ($F=3.814$, $P<0.01$) without modifying exploration of the familiar object, suggesting a short-term episodic memory impairment. Finally, object recognition was abolished with diazepam at 10 mg/kg.

Sequential spectral analysis of ECoG in rats. ECoG spectral analysis for each frequency band in the SM cortex showed that SR58611A was devoid of any effect in the energy of the delta, theta, alpha and beta bands, while diazepam increased the energy of the $\beta1$ and $\beta2$ bands at 3 mg/kg ($P<0.01$) (Table 7).

DISCUSSION

The main results of the present series of experiments show that the selective orally active and brain-penetrant β_3-adrenoceptor agonist, SR58611A, displays a behavioral profile that is consistent with an anxiolytic- and antide-

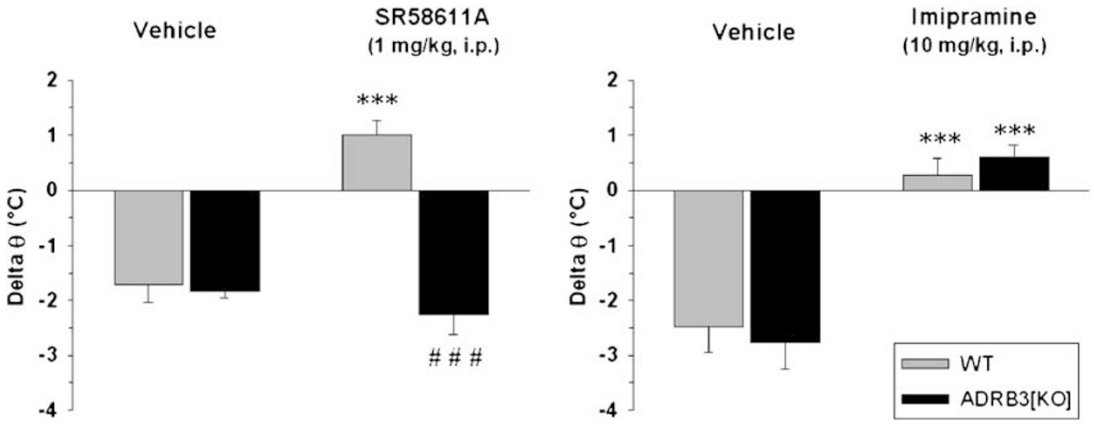

Figure 9 Effects of SR58611A and imipramine in ADRB3 KO and WT mice on apomorphine-induced hypothermia. Apomorphine (16 mg/kg, s.c.) was given 30 min after SR58611A (1 mg/kg, i.p.), imipramine (10 mg/kg, i.p.), or vehicle. Rectal temperature was measured 30 min after the apomorphine treatment. Data represent mean ± SEM, $^{***}P<0.001$, *vs* corresponding genotype groups; $^{\#\#\#}P<0.001$, *vs* same treatment in WT group in mice treated with apomorphine 16 mg/kg s.c., $N=8$–9.

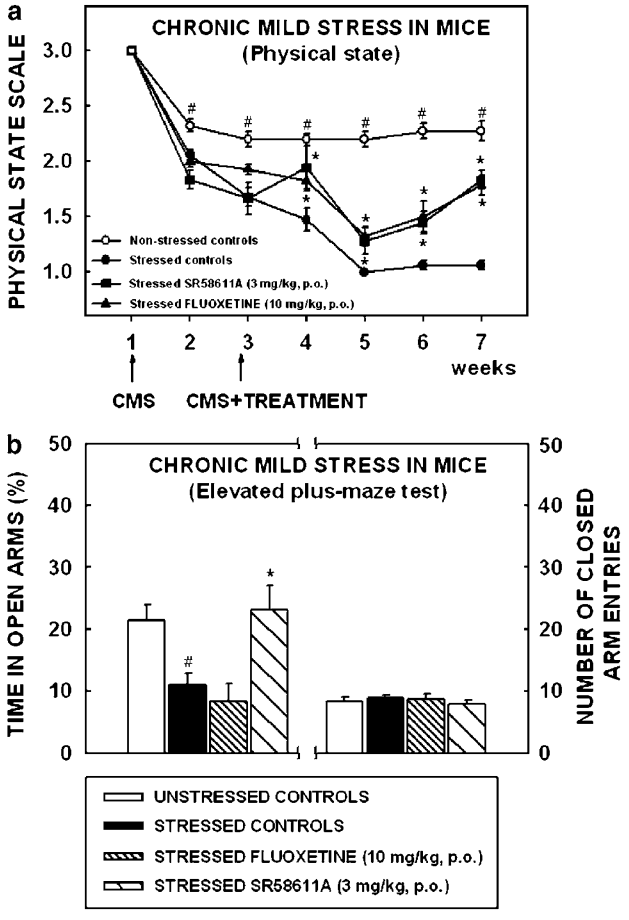

Figure 10 Effects of repeated administration of SR58611A and fluoxetine, (a) on the physical state of the coat in chronically stressed and non-stressed mice (data represent mean ± SEM, Wilcoxon $^{\#}P<0.01$, at least *vs* stressed controls, multiple comparison test $^{*}P<0.05$ at least *vs* stressed controls, $N=9$–20), and (b) on CMS-induced anxiogenic-like behavior in the elevated plus-maze test (data represent mean + SEM, Wilcoxon $\#P<0.05$ *vs* non-stressed controls or multiple comparison test, $^{*}P<0.05$ *vs* stressed controls, $N=9$–20). Administration of SR58611A (3 mg/kg) or fluoxetine (10 mg/kg) was started 2 weeks after the beginning of CMS. Animals were treated p.o. once a day until all experiments were completed (33 days).

pressant-like action (see Table 8). For the first time, behavioral effects of β_3-adrenoceptor stimulation were evaluated in a wide range of rodent models used for the screening of anxiolytics. When tested in classical models of anxiety, such as conflict paradigms (four-plate and punished drinking), the elevated plus-maze, and in social interaction procedures, SR58611A produced robust anxiolytic-like effects at concentrations from 0.3–10 mg/kg, depending on the model used. The difference in minimal effective doses between both elevated plus-maze experiments (ie, 0.3 *vs* 10 mg/kg) is unclear, but can be explained by the use of different species (ie, mouse *vs* rat), different experimental conditions (ie, stressed *vs* non-stressed animals) and/or different timing of administration (ie, 60 *vs* 90 min). The absence of significant alterations in the number of closed arm entries (a reliable measure of locomotor activity; Cruz *et al*, 1994) in the elevated plus-maze and in the distance traveled in the social interaction test in gerbils indicates that the anxiolytic-like activity was observed at doses that did not impair motor activity. Moreover, it is unlikely that the positive effects of SR58611A in the punished drinking test are due to decreased sensitivity to electric shocks since compounds that are endowed with analgesic properties, such as morphine, are inactive in conflict tests (present results; Griebel *et al*, 2002b; Vanover *et al*, 1999). It is important to note that the magnitude of the anxiolytic-like action of SR58611A in these models was overall comparable to that of the BZ anxiolytic diazepam, which was used as a positive control. Whether this may indicate a similar anxiolytic-like efficacy of β_3-adrenoceptor agonists compared to BZs; suggesting that these compounds may have a similar spectrum of therapeutic activity in anxiety disorders to BZs remains to be determined. Results obtained with SR58611A in the MDTB may, however, be relevant to this issue. The earlier MDTB studies have suggested that this procedure provides a model capable of responding to, and differentiating anxiolytic drugs of different classes through specific profiles of effect on different measures (Griebel and Sanger, 1999). Here, SR58611A modified significantly risk assessment, a behavior that has been shown to be particularly sensitive to BZs, that is, drugs used against generalized anxiety disorder

Table 4 Effects of SR58611A on Sleep/Wake Cycle Recorded during 6 h

	Dose mg/kg i.p.	W	SWS	REM	SWSlat	REMlat
SR58611A	3	−12	+12	−25	−19	+193
	10	+11	−3	−45**	+27	+392**
Fluoxetine	10	+34*	−6	−73*	+0.17	+198**

Data represent mean in % from control.
REM, rapid eye movement sleep; SWS, slow wave sleep, W, wakefulness.
*$P<0.05$, **$P<0.01$, 24 h after vehicle, $N=8$.

Table 5 Effects of SR58611A Alone or in Combination with Ethanol in the Rotarod Test

Compound	Dose	Time on rod (s)
	mg/kg, p.o.	
SR58611A	0	120.0±0.0
	3	120.0±0.0
	10	120.0±0.0
	30	120.0±0.0
	60	120.0±0.0
	100	120.0±0.0
Diazepam	0	120.0±0.0
	0.3	120.0±0.0
	1	113.9±4.1
	3	98.1±14.6
	10	42.6±17.2**
	30	23.0±12.2**
SR58611A	0+ethanol 20%	120.0±0.0
SR58611A	10+ethanol 20%	120.0±0.0
Diazepam	3+ethanol 20%	80.7±19.2*

Data represent mean±SEM.
*$P<0.05$, **$P<0.01$, $N=8$–10.

(GAD). It also produced clearcut effects on defensive aggression, a behavior that is claimed to be associated with certain aspects of stress disorders following traumatic events (Blanchard *et al*, 1997), thereby suggesting that SR58611A may be useful in these conditions and in GAD. In agreement with this idea are results from the social defeat stress-induced anxiety paradigm, where SR58611A completely antagonized the heightened emotionality in the elevated plus-maze test produced by prior (stressful) exposure to an aggressive resident. It is important to note that SR58611A produced anxiolytic-like effects in models sensitive to BZs but did not induce side effects typically observed with BZs, that is, motor impairment, sedation, interaction with ethanol, memory impairment, tolerance, and physical dependence.

To investigate potential antidepressant-like effects of SR58611A, we used two acute (the forced-swimming and the tonic immobility tests) and one chronic (the CMS paradigm) models of depression. In the forced-swimming test (Porsolt *et al*, 1977; Cryan *et al*, 2005), SR58611A produced dose-dependent antidepressant-like activity. These effects were comparable to those observed with the reference antidepressant, fluoxetine. The antidepressant potential of SR58611A was confirmed in the tonic immobility test, a paradigm that has been recently demonstrated to be sensitive to antidepressant treatments (Salomé *et al*, 2006). Importantly, in the CMS procedure (Griebel *et al*, 2002a, b; Sanchez *et al*, 2003), where the drug was given repeatedly for 33 days, SR58611A improved the degradation of the physical state of the coat of stressed animals. This finding suggests that SR58611A normalized grooming, an activity impaired by repeated stress. CMS caused the appearance of an 'anxious' profile, as was evidenced by the findings from the elevated plus-maze. This behavioral change was not seen in animals treated with SR58611A, indicating that the drug was able to prevent the stress-induced increase in anxiety levels. These results and the previous finding that SR58611A is able to restore a normal coping response when animals are exposed to inescapable aversive stimuli (Simiand *et al*, 1992) suggest that SR58611A may improve the behavioral consequences of an exposure to chronic stress conditions suggesting a restored cognitive flexibility. Overall, the effects of SR58611A in the CMS paralleled those of repeated treatment with fluoxetine. Based on the well-accepted predictive validity of the CMS (Willner *et al*, 1992), the present findings indicate that SR58611A has antidepressant-like properties that are comparable in terms of efficacy of the effects to those of fluoxetine. Supporting these behavioral data, our electroencephalographic results show that SR58611A modified spontaneous sleep parameters in a manner comparable to that observed with fluoxetine. Both compounds profoundly reduced REM quantity and increased latency. Of particular interest, increased REM quantity in human is a characteristic of the depressed phenotype, and the ability to suppress REM sleep is considered as a common feature of drugs with antidepressant properties (Sharpley and Cowen, 1995).

In the absence of selective and centrally acting β_3-adrenoceptor antagonist, the contribution of β_3 adrenoceptors in the anxiolytic- and antidepressant-like actions of SR58611A cannot be pharmacologically addressed. Flumazenil, propranolol, ICI-118,551, betaxolol, and *p*-CA did not block the activity of SR58611A in the punished drinking test thereby indicating that anxiolytic-like properties of SR58611A are at least not mediated by a direct action on β_1 or β_2 adrenoceptors, $GABA_A$/BZ receptor subtypes, or the 5-HT transporter. Importantly, our findings that the inhibitory effects of SR58611A on autonomic response to a predator odor stress and apomophine-induced hypothermia

Table 6 Effects of SR58611A on Spatial Reference Memory in the Water Maze and on Visual Episodic Memory in the Object Recognition Tests in Rats

		Morris water maze			Object recognition		
Compound	**Dose (mg/kg)**	**Latency on day 1 (s)**	**Latency on day 2 (s)**	**Latency on day 3 (s)**	**Locomotor activity (s)**	**Exploration duration (s) familiar object**	**Exploration duration (s) new object**
SR58611A	0	76.8±10.5	60.7±11.5	44.4±12.8***	26.7±2.4	7.5±0.9	18.2±2.2***
	1	60.6±6.9	36.4±6.9**	26.0±4.6***	—	—	—
	3	68.0±8.3	47.3±8.3*	48.6±10.8**	24.2±1.6	8.1±1.2	17.0±1.1***
	10	69.4±7.1	40.9±6.8***	42.1±8.6***	27.0±1.4	7.2±1.1	18.3±1.5***
	30	—	—	—	26.2±1.8	6.8±1.5	18.1±1.3***
Diazepam	0	76.8±8.4	69.9±12.1	42.2±11.9**	23.5±1.5	9.9±0.9	19.5±2.0***
	1	94.9±9.5	91.9±8.5	54.0±14.8***	22.4±1.2	8.9±1.0	14.3±1.6**$^{\#}$
	3	84.8±11.9	81.8±10.0	78.3±11.7	21.8±1.1	8.6±1.4	12.3±1.5*$^{\#}$
	10	96.5±9.8	98.6±8.6	97.7±10.0	19.2±0.8*	6.5±0.8	7.5±1.2$^{\#\#\#}$

Data represent mean±SEM.
*$P<0.05$, **$P<0.01$, ***$P<0.01$ *vs* day 1 or familiar object, $^{\#}P<0.05$, $^{\#\#\#}P<0.001$ *vs* control group in the object recognition task, $N=9$–10.

Table 7 Effects of SR58611A on ECoG Spectral Analysis in the Sensorimotor Cortex

Compound	**Dose (mg/kg i.p.)**	**Delta band**	**Theta band**	**Alpha 1 band**	**Alpha 2 band**	**Beta 1 band**	**Beta 2 band**	**Total power**
SR58611A	0	42.51±2.23	25.96±0.97	11.93±0.67	6.68±0.51	7.90±0.59	4.46±0.25	100.0±0.0
	3	46.74±1.94	22.97±0.70	10.53±0.36	7.60±0.54	8.41±0.77	3.75±0.20	100.0±0.0
Diazepam	3	34.8±2.0	23.4±1.1	11.6±0.5	8.4±1.0	13.6±0.5**	6.8±0.4**	100.0±0.0

Data represent mean±SEM in min.
**$P<0.01$, Student, $N=5$–6.

Table 8 Summary of the Effects of SR58611A in Rodents (Minimal Effective Dose or MED)

	MED p.o. or (i.p.)		
Tests	**SR58611A**	**Diazepam or chlordiazepoxide**	**Fluoxetine or imipramine**
Four-plate in gerbils	3	(1)	
Punished drinking in rats	(3)	(3)	
Elevated plus maze in rats	0.3	3	
Social interaction in gerbils	1	0.1	
Social interaction in mice	1	1.5	
Mouse defense test battery	1	1	
Social defeat in mice	10	1	
Stress-induced hyperthermia in mice	3	10	
Forced swimming in rats	1		3
Tonic immobility in gerbils	3		(7.5)
Chronic mild stress in mice	3		10
Sleep/waking cycle in rats	(10)		(10)
Apomorphine-induced hypothermia in mice	(1)		(10)
Rotarod in mice	IN	10	
Interaction with ethanol in mice	IN	3	
Morris water maze in rats	IN	(3)	
Withdrawal-induced anxiety in mice	IN	(2.5)	
Object recognition in rats	IN	1	
ECoG spectral analysis in rats	IN	(3)	

IN, inactive; MED, minimal effective dose.
Comparison with reference compounds (BZ, diazepam, SSRI, fluoxetine or tricyclic, imipramine).

were no longer observed in mice lacking the β_3 adrenoceptor provide evidence that the anxiolytic- and the antidepressant-like action of the compound involves β_3 adrenoceptors.

The neurochemical mechanisms underlying SR58611A activities reported here remain to be fully elucidated. With respect to sleep parameters, a large body of evidence support the notion that many antidepressant drugs reduce REM sleep through facilitation of NA and/or serotonergic function (Sharpley and Cowen, 1995). Moreover, the 5-HT precursor, tryptophan, was shown to reduce REM sleep in rat (Bakalian and Fernstrom, 1990), its brain concentrations being increased following β_3-adrenoceptors stimulation (Lenard *et al*, 2003). The idea that SR58611A activates 5-HT and NA transmission is strenghtened by recent findings from this laboratory showing that the drug increases 5-HT and NE, but not dopaminergic neurotransmission in several brain areas in rats (Claustre *et al*, Submitted). More specifically, the drug increased the synthesis of 5-HT and tryptophan levels in the cortex, hippocampus, hypothalamus, and striatum, and the release of 5-HT in the prefrontal cortex. Interestingly, the effect of SR58611A on 5-HT transmission was no longer observed in mice lacking the β_3 adrenoceptor, indicating that the activation of β_3 adrenoceptors is necessary to stimulate 5-HT transmission. Moreover, SR58611A increased the release of NE in the prefrontal cortex, hippocampus, and hypothalamus and the firing rate of NE neurons in the locus coeruleus. Altogether, the present findings suggest that SR58611A will serve as a useful tool for investigating the role of β_3 adrenoceptors in emotional processes, and its large spectrum of activity in animal models of anxiety and depression suggests that the selective stimulation of β_3 adrenoceptors may represent an innovative approach in the management of anxiety and depressive disorders.

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