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$GABA_A$–benzodiazepine receptor complex sensitivity in 5-HT_{1A} receptor knockout mice on a 129/Sv background

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Abstract

Previous studies in 5-HT_{1A} receptor knockout (1AKO) mice on a mixed Swiss Webster×129/Sv (SW×129/Sv) and a pure 129/Sv genetic background suggest a differential γ-aminobutyric acid ($GABA_A$)–benzodiazepine receptor complex sensitivity in both strains, independent from the anxious phenotype. To further investigate these discrepancies, various $GABA_A$–benzodiazepine receptor ligands were tested in different behavioral paradigms in 1AKO and wild type (WT) mice on a 129/Sv background. 1AKO and WT mice responded comparably to alprazolam, flumazenil, alcohol and pentylenetetrazol as measured in the stress-induced hyperthermia paradigm. In addition, sedative–anesthetic effects of pentobarbital measured via the righting reflex were similar and a selected dose of diazepam exerted similar anxiolytic effects in both genotypes in the elevated plus maze. In conclusion, 1AKO mice on a 129/Sv background have undisturbed $GABA_A$–benzodiazepine receptor sensitivity in contrast to those described on a mixed Swiss Webster×129/Sv background. The anxious phenotype of 1AKO mice seems to occur independent of the $GABA_A$–benzodiazepine receptor complex functioning.

Keywords: 5-HT_{1A} receptor knockout mice; $GABA_A$–benzodiazepine receptor; (In vivo); Pentylenetetrazol; Positive righting reflex; Stress-induced hyperthermia

1. Introduction

Among the 14 different 5-HT receptors in the brain (Hoyer et al., 1994), the 5-HT_{1A} receptor is particularly thought to play an important role in the etiology of anxiety and depression (Deakin, 1993; De Vry, 1995; Olivier et al., 1999). To study the putative contribution of 5-HT_{1A} receptors to anxiety, mice lacking 5-HT_{1A} receptors have been generated in different genetic backgrounds and it has been shown that under certain conditions, these mice display enhanced anxiety compared to their corresponding wild types (WT) (for reviews: Gingrich and Hen, 2001; Olivier et al., 2001).

Recently, it has been suggested that the underlying mechanism of the anxious phenotype of 5-HT_{1A} receptor knockout mice on a mixed Swiss Webster×129/Sv (SW×129/Sv) background is a deviant γ-aminobutyric acid ($GABA_A$)–benzodiazepine receptor complex (Sibille et al., 2000). In that study, downregulation of both $GABA_A$ α_1 and α_2 receptor subunits, as well as a benzodiazepine-resistant anxiety in the elevated plus maze in knockout mice was reported. Interestingly, in 5-HT_{1A} receptor knockout mice on a pure 129/Sv background, pharmacological studies suggest an unaltered $GABA_A$–benzodiazepine receptor complex in the stress-induced hyperthermia paradigm (Pattij et al., 2001, 2002), supported by behavioral observations (Gross et al., 2000). Despite the preliminary observed differences in the $GABA_A$–benzodiazepine receptor complex, knockout mice on a mixed Swiss Webster×129/Sv background (Parks et al., 1998; Sibille et al., 2000), as well as those on a pure 129/Sv background (Gross et al., 2000; Pattij et al., 2002; Ramboz et al., 1998; Zhuang et al., 1999), display enhanced anxiety compared to their corresponding

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wild types under certain conditions. Consequently, this could lead to the assumption that the anxious phenotype of knockout mice on a 129/Sv background appears to be independent of changes in $GABA_A$–benzodiazepine receptor sensitivity.

To further investigate $GABA_A$–benzodiazepine receptor sensitivity in knockout mice on a pure 129/Sv genetic background, effects of various $GABA_A$–benzodiazepine receptor-modulating drugs were assessed in different benzodiazepine-sensitive paradigms.

In the previous findings with diazepam in the stress-induced hyperthermia paradigm, a simple animal model to test putative anxiolytic properties of drugs (Borsini et al., 1989; Van der Heyden et al., 1997) showed that diazepam had comparable anxiolytic effects in knockout compared to wild type mice (Pattij et al., 2001, 2002). First, to extend these results, effects of alprazolam, alcohol, flumazenil, a nonselective $GABA_A$–benzodiazepine receptor antagonist and pentylenetetrazol were assessed in the stress-induced hyperthermia paradigm. Second, sensitivity of knockout mice to the anesthetic effects of the barbiturate pentobarbital on righting reflex was studied in order to replicate earlier findings showing decreased sensitivity in knockout mice on a mixed Swiss Webster×129/Sv background to the anesthetic effects of pentobarbital (Sibille et al., 2000). To exclude the possibility that the population of $GABA_A$–benzodiazepine receptors playing a role in the stress-induced hyperthermia paradigm and/or righting reflex differs from the population playing a role in behavioral paradigms, the anxiolytic effect of a selected dose of diazepam in the elevated plus maze was also determined.

2. Materials and methods

2.1. Subjects

Male homozygote 5-HT_{1A} receptor knockout and wild type mice were bred within the laboratory animal facilities of the Utrecht University (Gemeenschappelijk dierenlaboratorium, Utrecht, Netherlands). The breeding founders were originally obtained from Dr. R. Hen (Columbia University, New York, USA) and were derived from established colonies from the 129/Sv strain (Ramboz et al., 1998). Mice were generated by breeding homozygote knockout and wild type mice with the same 129/Sv genetic background.

At the start of the experiments, animals were 8 weeks old and were housed in same-genotype groups of eight animals per cage (43×26×15 cm) under nonreversed 12 h light–12 h dark cycle conditions (lights on from 7:00 to 19:00 h). The animals were housed at controlled room temperature (21±2 °C) and relative humidity of 60±15% with standard rodent food pellets (Hope Farms, Woerden, Netherlands) and water freely available during the whole experiment. For the stress-induced hyperthermia experiments on the afternoon (between 15:30 and 16:30 h) of the day prior to an experiment, mice were housed individually in smaller cages (23.5×13.5×13 cm). After completion of a stress-induced hyperthermia experiment animals were regrouped in the same group. Stress-induced hyperthermia and elevated plus maze experiments were carried out between 9:00 and 12:00 h at least 1 week after habituation to the laboratory. Different mice were used for stress-induced hyperthermia and elevated plus maze experiments. Pentobarbital experiments were conducted in mice that were previously used in the elevated plus maze. All experiments were carried out with the approval of the Animal Experiments Ethics Committee of the Faculties of Pharmaceutical Sciences, Chemistry and Biology of the Utrecht University.

2.2. Procedure

2.2.1. Stress-induced hyperthermia

The stress-induced hyperthermia experiments were performed as described previously (Van der Heyden et al., 1997). Genotypes (wild type or knockout) were randomly assigned to cages and drug doses in each experiment. The temperature of the mice was measured by inserting a thermistor probe for a length of 2 cm into the rectum. Digital recordings were obtained with an accuracy of 0.1 °C using a Tegam 871A digital thermometer (Geneva, OH, USA). The probe, dipped into silicon oil before insertion, was held in the rectum until a stable rectal temperature had been obtained for at least 10 s. On a test day, mice were injected subcutaneously with either drug or vehicle 60 min before the first temperature measurement ($T1$). The temperature was again measured 10 min later ($T2$). Per test day, a total of 40 mice were tested.

2.2.2. Positive righting reflex

Mice received a single intraperitoneal dose of pentobarbital (60 mg/kg) and were continuously monitored during the anesthesia. During anesthesia, mice were positioned on their back in clean cages that were placed on heating pads. A positive righting reflex was scored when animals were able to turn upright three times within a 30-s period. After the first spontaneous turn of the animal, the experimenter replaced the animal on its back.

2.2.3. Elevated plus maze

The elevated plus maze (black Plexiglas floor and walls) consisted of two closed and two open black arms (30×5 cm; walls of closed arms: 15 cm high) radiating from a common center platform (5×5 cm) and elevated 100 cm above floor level. The animals were tested during the animals' light phase between 9:00 and 14:00 h, but during testing, the maze was dimly illuminated by a red light (5 lx). Mice were housed in same-genotype groups of eight animals per cage and were randomly allocated to one selected diazepam dose (based on previous experiments) or vehicle and tested in

counterbalanced order. Testing started 30 min after an intraperitoneal diazepam injection by placing a mouse on the center platform facing an open arm. The test lasted 10 min, and between tests, the maze was first thoroughly cleaned with water and subsequently with 70% alcohol. Behavior of the mice was scored online with a tracking system (Ethovision®, Noldus, Wageningen, Netherlands).

2.3. Data analysis

2.3.1. Stress-induced hyperthermia

In each individual mouse, a basal 'nonstressed' temperature 60 min after drug treatment ($T1$), and a 'stressed' temperature 70 min after drug treatment ($T2$) was determined. Separately, stress-induced hyperthermia (ΔT) was determined as follows: $\Delta T = T2 - T1$. Data were analysed by univariate analysis of variance (ANOVA) with temperature measurements as within-subject variable and genotype and drug dose as between-subjects variables. When appropriate, further post hoc comparisons were made using Bonferroni t-tests.

2.3.2. Positive righting reflex

Latency (in min) until regaining the righting reflex was determined for each mouse. Data were analysed by means of univariate ANOVA with latencies as within-subject variable and genotype as between-subjects variable.

2.3.3. Elevated plus maze

The number of open arm entries and percentage time spent in the open arms were scored and analysed by means of univariate analysis of variance with open arm entries/percentages as within-subject variable and genotype and drug dose as between-subjects variable.

All statistical analyses were performed using the Statistical Package for the Social Sciences version 9.0 (SPSS, Chicago, USA) and in case of statistically significant dose and/or genotype effects, data were further analyzed with a univariate analysis of variance. The level of significance was $P < 0.05$ for all tests.

2.4. Drugs

Pentylenetetrazol (RBI, Natick, USA) was dissolved in 0.9% physiological saline. Alprazolam (Upjohn, Kalamazoo, USA), diazepam (Brunschwig Chemie, Amsterdam, Netherlands) and flumazenil (Roche Nederland, Mijdrecht, Netherlands) were suspended in a 0.5% gelatin–5% mannitol solution. Alcohol at 96% (Merck, Darmstadt, Germany) was diluted in water. Alprazolam and alcohol were administered orally (p.o.); flumazenil and pentylenetetrazol were administered subcutaneously (s.c.); diazepam and pentobarbital (Nembutal®, Sanofi Sante Animale Benelux, Maassluis, Netherlands) were administered intraperitoneally (i.p.). All drug solutions were freshly prepared each test day and injected in a volume of 10 ml/kg.

3. Results

3.1. Stress-induced hyperthermia

3.1.1. Stress-effects and basal temperature over experiments

Across successive experiments (alprazolam, flumazenil, alcohol and pentylenetetrazol, in that sequence), the increase of approximately 1.5 °C in temperature due to the rectal procedure (ΔT) was highly significant [$F(1,72) = 226.49$, $P < 0.001$]. This effect was not different in knockout mice compared to wild type mice (no genotype × stress interaction effect). In addition, there were neither experiment nor experiment × stress effects, indicating that the stress response did not change over experiments and was similar in all experiments. Basal 'non-stressed' rectal temperature, $T1$, was different between genotypes at vehicle conditions across successive experiments, although the effect was small [$F(1,80) = 4.16$, $P = 0.042$] with neither a significant experiment nor experiment × stress effect.

Further comparisons indicated that only in the alcohol experiment 5-HT_{1A} receptor knockout (1AKO), mice had a higher basal rectal temperature compared to WT mice.

3.1.2. Alprazolam

Only the highest dose of alprazolam decreased $T1$ [$F(3,80) = 14.63$, $P < 0.001$] with no genotype or genotype × dose interaction effects, indicating that the high-dose alprazolam in both genotypes caused comparable hypothermia. The rectal temperatures after stress, $T2$, were dose-dependently affected by alprazolam [$F(3,80) = 49.12$, $P < 0.001$] and post hoc analyses indicated that the intermediate and the high doses of alprazolam decreased $T2$ (1.0 and 3.0 mg/kg, respectively) compared to vehicle, with neither genotype nor genotype × dose interaction effects. All doses of alprazolam (0.3, 1.0 and 3.0 mg/kg) significantly antagonized the stress-induced hyperthermia (ΔT) compared to vehicle [$F(3,80) = 33.18$, $P < 0.001$], with no genotype effect, but with a genotype × dose interaction effect [$F(3,80) = 3.07$, $P < 0.05$] (Fig. 1A).

3.1.3. Flumazenil

Flumazenil had no effect on $T1$, $T2$ or ΔT. Also, there were neither genotype effects nor genotype × dose interaction effects (Fig. 1B).

3.1.4. Alcohol

Alcohol decreased $T1$ [$F(3,80) = 3.12$, $P < 0.05$] with a genotype effect [$F(1,80) = 19.22$, $P < 0.001$], but no genotype × dose interaction effect. Further analyses showed that the high dose of alcohol (4000 mg/kg) decreased $T1$, but only in knockout mice. Moreover, $T1$s were higher in knockout compared to wild type mice at vehicle and the two intermediate doses of alcohol (Fig. 1C).

Alcohol also decreased $T2$ [$F(3,80) = 20.08$, $P < 0.001$] with genotype and genotype × dose interaction effects

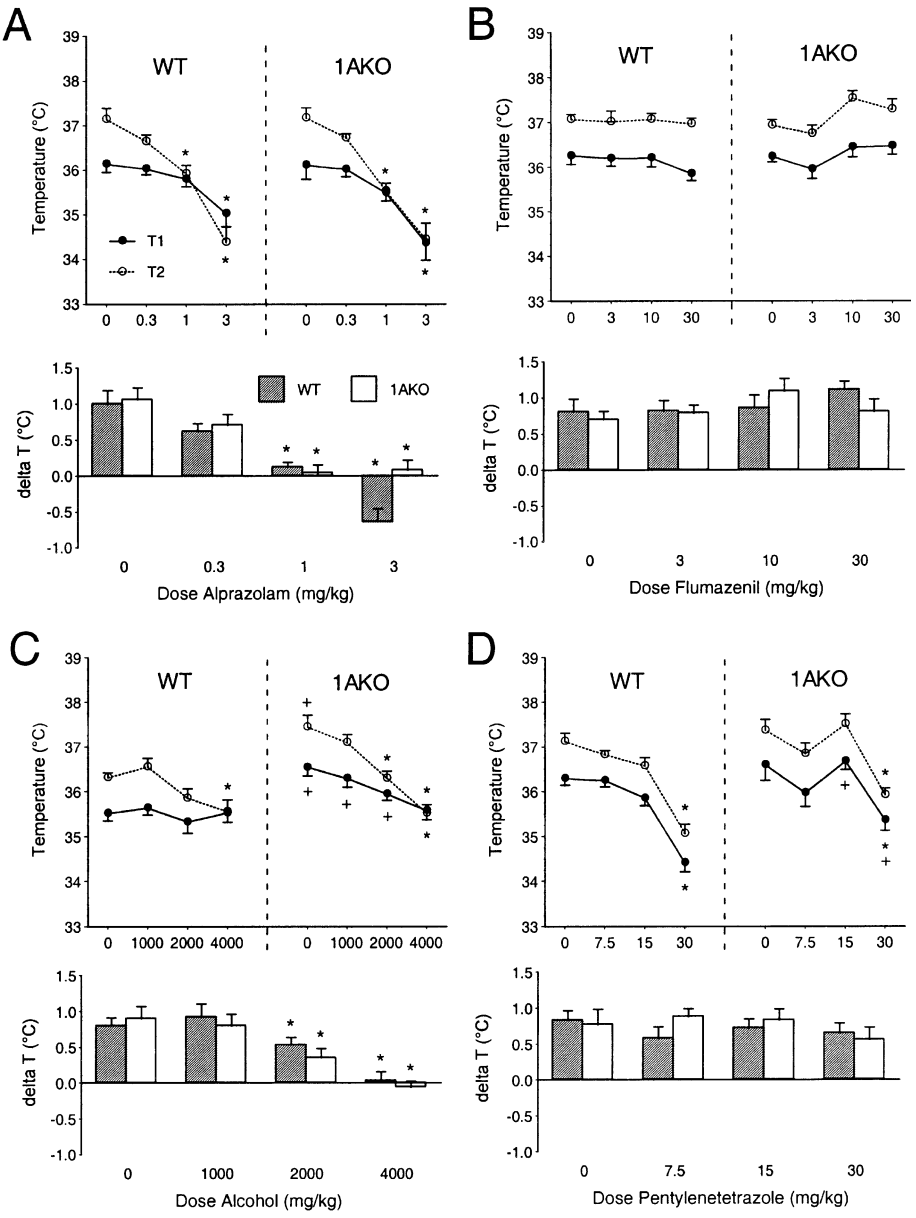

Fig. 1. Effects of (A) alprazolam, (B) flumazenil, (C) alcohol and (D) pentylenetetrazole in the stress-induced hyperthermia paradigm on the first rectal temperature measurement $T1$ (mean±S.E.M.; solid circles) and the second temperature measurement $T2$ (open circles) in 129/Sv wild type (left top panels) and 1AKO (right top panels) mice (n=10 per dose and genotype). The bottom panel of each subfigure (A–D) shows ΔT for the WT (hatched bars) and 1AKO mice (white bars). *$P<0.05$ compared to vehicle within genotype. +$P<0.05$ wild type compared to knockout mice.

[$F(1,80)=12.96$, $P<0.005$ and $F(3,80)=2.80$, $P<0.05$, respectively]. In wild type mice, the 4000-mg/kg dose decreased $T2$, whereas in knockout mice the 2000- and 4000-mg/kg doses of alcohol decreased $T2$. Moreover, knockout mice displayed a higher $T2$ at the vehicle condition compared to wild type mice.

The 2000- and 4000-mg/kg doses of alcohol antagonized the stress-induced hyperthermia [$F(3,80)=18.16$, $P<0.001$], with neither genotype nor genotype×dose interaction effects, indicating that the anxiolytic properties of alcohol were comparable in both genotypes.

3.1.5. Pentylenetetrazol

The high dose (30 mg/kg) of pentylenetetrazol decreased $T1$ in both knockout and wild type mice [$F(3,80)=19.39$, $P<0.001$]. Moreover, $T1$s were higher in knockout mice compared to wild type mice at 15 and 30 mg/kg pentylenetetrazol, respectively [genotype effect: $F(1,80)=7.68$, $P<0.01$] (Fig. 1D). Similarly, pentylenetetrazol also decreased $T2$ in both knockout and wild type mice at the high dose [$F(3,80)=32.13$, $P<0.001$], and in addition, at the 15- and 30-mg/kg doses of pentylenetetrazol, stressed temperatures were higher in knockout mice than in wild type mice

[genotype effect: $F(1,80) = 13.29$, $P < 0.001$]. Pentylenetetrazol did not block stress-induced hyperthermia at any dose. Moreover, there were neither genotype nor genotype × dose interaction effects.

3.2. Positive righting reflex

Latencies until regaining the righting reflex did not differ between wild type (184 min) and 5-HT_{1A} receptor knockout mice (173 min) [$F(1,22) = 0.353$, $P = 0.56$, NS].

3.3. Elevated plus maze

Fig. 2 shows that the single selected dose of diazepam (1.0 mg/kg, i.p.) significantly increased the total number of open arm entries and total time spent in the open arms [$F(1,44) = 5.03$, $P < 0.05$ and $F(1,44) = 5.99$, $P < 0.05$, respectively], but there was neither an overall genotype nor genotype × dose interaction effect [$F(3,39) = 1.56$, $P = 0.214$, NS and $F(3,39) = 0.402$, $P = 0.750$, NS, respectively). Total locomotor activity in the elevated plus maze under vehicle conditions also did not differ between genotypes [$F(1,44) = 0.193$, $P = 0.663$, NS].

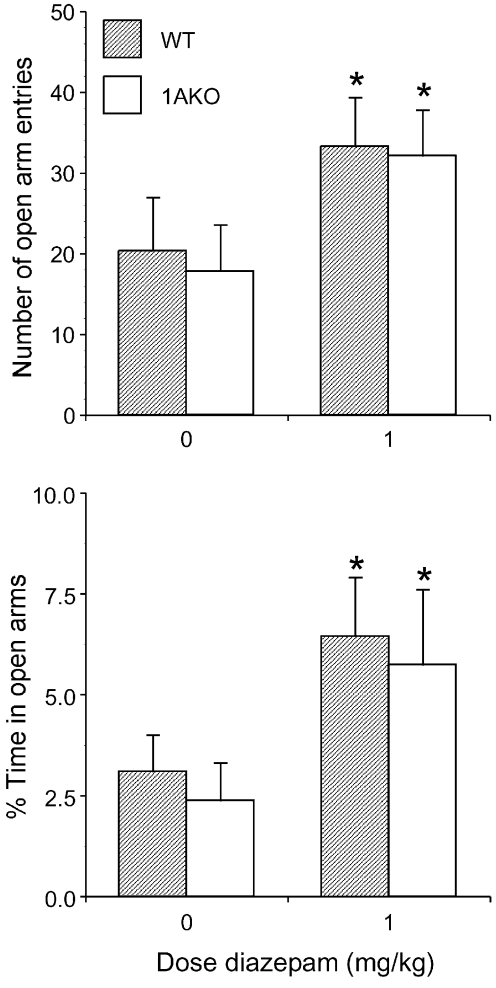

Fig. 2. Effects of a single dose of diazepam in the elevated plus maze on the number of open arm entries (upper panel) and percentage time spent on open arms (lower panel) in 129/Sv wild type and 1AKO mice (wild type mice: vehicle dose n=10, single diazepam dose n=12; 1AKO mice: vehicle dose n=12, single diazepam dose n=11). All data are mean ± S.E.M.

4. Discussion

It is well established that $GABA_A$ receptors are ligand-gated chloride ion channels and consist of five receptor subunits derived from four subunit families, namely: α_{1-6}, β_{1-3}, γ_{1-3} and δ (for reviews: e.g. Mehta and Ticku, 1999; Sieghart et al., 1999). Depending on their subunit composition, $GABA_A$ receptors display different affinities for various ligands, including barbiturates, benzodiazepines, ethanol and neurosteroids (reviewed in Johnston, 1996; Lüddens et al., 1995).

Recently, it was shown that the α_1 subunit is mainly responsible for the sedative effects of the benzodiazepine receptor agonist diazepam (Rudolph et al., 1999) and the α_2 subunit for the anxiolytic effects of diazepam (Löw et al., 2000).

Previous results in 5-HT_{1A} receptor knockout (1AKO) mice on a mixed Swiss Webster × 129/Sv (SW × 129/Sv) background showed that these mice have an altered $GABA_A$–benzodiazepine receptor complex, mainly expressed by downregulation of α_1 and α_2 subunits of the $GABA_A$ receptor in the amygdala, hippocampus and cortex (Sibille et al., 2000). Initial studies with diazepam in 1AKO mice on a pure 129/Sv background showed comparable anxiolytic activity of diazepam in 1AKO and wild type mice (Pattij et al., 2001, 2002). This suggests that the α_2 subunit is, at least functionally, fully activated in 1AKO mice in this background. However, diazepam is a full $GABA_A$–benzodiazepine receptor agonist and does not discriminate between the various $GABA_A$ receptor subunits; therefore, it is difficult to conclude on the basis of experiments with diazepam alone that a certain receptor subunit is differentially affected in 1AKO mice in vivo.

The present study elaborated further on characterizing $GABA_A$–benzodiazepine receptor complex functions in 1AKO mice on a pure 129/Sv background. Results indicate that various ligands differentially targeted at the $GABA_A$–benzodiazepine receptor complex have comparable in vivo effects in WT and 1AKO mice.

First, previous findings with diazepam in the stress-induced hyperthermia paradigm were extended (Pattij et al., 2001, 2002) and to this end, a variety of benzodiazepine receptor ligands was tested in the stress-induced hyperthermia paradigm. Effects of the full benzodiazepine receptor agonist, the triazolobenzodiazepine alprazolam (Dawson et al., 1984), were studied in the stress-induced hyperthermia paradigm. Results show that on basal temperature, the highest dose of alprazolam (3 mg/kg, p.o.) caused comparable hypothermia in both genotypes. Moreover,

alprazolam blocked stress-induced hyperthermia in WT mice in line with previous reports showing anxiolytic activity of benzodiazepine receptor agonists in the stress-induced hyperthermia paradigm in various strains of mice (Olivier et al., 2002; Zethof et al., 1995). More importantly, stress-induced hyperthermia was equally antagonized by alprazolam in 1AKO mice compared to WT mice, suggesting that the anxiolytic activity of alprazolam is intact in 1AKO mice on a 129/Sv background.

In addition to full benzodiazepine receptor agonists, the nonselective benzodiazepine receptor antagonist flumazenil (Brogden and Goa, 1991) was tested in the stress-induced hyperthermia paradigm. Although in previous studies, flumazenil had no effects in the stress-induced hyperthermia paradigm in NMRI mice (Olivier et al., 2002), possibly in 1AKO mice with a putatively altered $GABA_A$–benzodiazepine receptor complex, flumazenil may have anxiety-modulating effects. Support for this comes from a recent study showing that flumazenil had anxiolytic effects in BALB/c, but not in C57BL/6 mice, possibly caused by strain-specific differences in the $GABA_A$–benzodiazepine receptor complex (Belzung et al., 2000). Unfortunately, in the present study, flumazenil did not have any effect on either basal temperature or stress-induced hyperthermia in either genotype.

Some pharmacological actions of alcohol are similar to those of benzodiazepines and barbiturates, and because of that, several effects of alcohol are thought to be mediated through $GABA_A$ receptors (Mehta and Ticku, 1999). Alcohol putatively interacts with a non-benzodiazepine allosteric site at the $GABA_A$ receptor complex (Rabow et al., 1995) and in previous experiments has been shown to block stress-induced hyperthermia (Olivier et al., 2002; Zethof et al., 1995). In the present experiment, alcohol decreased basal temperature at the highest dose (4000 mg/kg, p.o.), but only in 1AKO mice, suggesting that 1AKO mice were more sensitive to the hypothermic effects of alcohol. Nonetheless, stress-induced hyperthermia as a measure of anxiety was dose-dependently and similarly antagonized in both WT and 1AKO mice.

Lastly, pentylenetetrazol was tested in the stress-induced hyperthermia paradigm, and although its mechanism of action is not yet fully understood, pentylenetetrazol is thought to act at the picrotoxin site of the $GABA_A$ receptor. Pentylenetetrazol inhibits GABA-activated ion channels and has convulsant properties (Huang et al., 2001; Johnston, 1996). Moreover, pentylenetetrazol is often used as an anxiogenic compound, and its effects are thought to represent an animal analogue of human anxiety (Lal and Emmett-Oglesby, 1983). Consequently, hyperthermic effects of pentylenetetrazol were expected on basal temperatures. Surprisingly, the highest dose of pentylenetetrazol (30 mg/kg, s.c.) decreased both basal temperature and stressed rectal temperature ($T2$) in both genotypes equally, but did not antagonize the stress-induced hyperthermia. It is difficult to explain the present findings, but it has been previously shown that anxiogenic properties of drugs are difficult to detect in the stress-induced hyperthermia paradigm (Zethof et al., 1995).

In summary, the various $GABA_A$–benzodiazepine receptor ligands in the stress-induced hyperthermia paradigm did not have differential effects in 1AKO compared to WT mice on a pure 129/Sv background, contrasting the findings obtained in 1AKO mice on a SW×129/Sv background (Sibille et al., 2000). Therefore, in addition to the stress-induced hyperthermia experiments, pentobarbital-induced anaesthesia was studied, in analogy to Sibille et al. (2000).

Barbiturates are used as sedative–hypnotics, anticonvulsants and anaesthetics, and have been shown to enhance the activation of $GABA_A$ receptors. In contrast to the actions of benzodiazepines, which appear to be restricted to particular $GABA_A$ receptors, the actions of barbiturates are more widespread and less restricted (Johnston, 1996). Probably, the enhancement of activation of $GABA_A$ receptors causes the sedative–hypnotic and anaesthetic actions of barbiturates (Johnston, 1996). In $5\text{-}HT_{1A}$ receptor knockout mice on the mixed SW×129/Sv background, the sedative–hypnotic effects of pentobarbital are less pronounced compared to their corresponding WT mice (Sibille et al., 2000). In the present study, however, the effects of pentobarbital-induced anaesthesia were not different between genotypes in latencies to regain the righting reflex. Thus, taken together, results obtained in the stress-induced hyperthermia experiments and righting reflex paradigm reveal no abnormalities in $GABA_A$–benzodiazepine receptor function in 1AKO compared to WT mice on a 129/Sv background, as evidenced by comparable in vivo sensitivity to a variety of benzodiazepine receptor ligands.

Notwithstanding these findings, one could still argue that the population of $GABA_A$–benzodiazepine receptors playing a role in these paradigms differs from the ones playing a role in the anxiolytic activity of benzodiazepines in, for instance, the elevated plus maze. Although little is still known about the exact brain mechanisms activated upon stress-induced hyperthermia stress, preliminary data obtained with Fos-immunoreactivity show that both brain areas involved in thermoregulation are activated, as well as other areas that are activated upon, e.g. restraint stress, acute swim stress, painful stimuli and even elevated plus maze exposure (Veening et al., unpublished data).

To exclude the possibility of activation of different populations of $GABA_A$–benzodiazepine receptors in the various paradigms, a single selected dose of diazepam (1 mg/kg, i.p.) was tested in the elevated plus maze in WT and 1AKO mice. In addition to the stress-induced hyperthermia and righting reflex data, the single selected dose of diazepam exerted comparable anxiolytic activity in the elevated plus maze in both WT and 1AKO mice on a pure 129/Sv background, indicating that no major shift in the dose–response curve for anxiolytic activity is found. It should be noted though that under vehicle conditions, 1AKO mice did not show elevated anxiety compared to wild type mice, contrasting initial reports showing an anxious phenotype of

1AKO mice in the elevated plus and elevated zero maze (Heisler et al., 1998; Parks et al., 1998; Ramboz et al., 1998). One possible explanation for these findings could be that behavior in approach–avoidance paradigms is very sensitive to environmental variations and as such is not easy to replicate (Crabbe et al., 1999). Moreover, putative anxiogenic and anxiolytic effects obtained in the elevated plus maze are often confounded with changes in locomotor activity (Dawson and Tricklebank, 1995). Compared to other inbred mouse strains, the 129/Sv strain has been shown to be one of the least explorative and most anxious strains in several approach–avoidance paradigms (Paulus et al., 1999; Voikar et al., 2001). These low levels of exploration might therefore hamper the expression of a phenotypic effect.

Altogether, the elevated plus maze data support the other results obtained in this study: that the $GABA_A$–benzodiazepine receptor complex functioning, as measured in vivo with pharmacological tools, is intact in 5-HT_{1A} receptor knockout mice made on a pure 129/Sv background. Although in the present study, no 'anxious' phenotype could be found in the elevated plus maze, under certain conditions (novelty, injection-stress), these mice did have a clear anxious phenotype (Olivier et al., 2001; Pattij et al., 2002). Apparently, the anxious phenotype of 5-HT_{1A} receptor knockout mice on a pure 129/Sv background is not necessarily caused by a deficient $GABA_A$ receptor complex. Whether the deficient $GABA_A$ receptors in the SW×129/Sv 1AKO mice causes the anxious phenotype should be further investigated. It is surprising that differences in genetic background of the two lines of 5-HT_{1A} receptor knockout mice may lead to differential functioning of certain $GABA_A$ receptors. Recent studies stress the importance of genetic background and show that the expression of a knockout phenotype can be greatly influenced by the genetic background (see for example: Paradee et al., 1999; Dobkin et al., 2000; Wolfer and Lipp, 2000). However, knocking out a particular gene (in this case, the 5-HT_{1A} receptor gene) which may lead to disturbance of a neurotransmitter system in one genetic background and not in the other, is not previously observed. That such a thing might happen is, of course, not completely surprising, because major differences exist between inbred mouse strains regarding endogenous traits and behavior (Crawley et al., 1997). Whether the present results can be explained by the difference in genetic background is currently under investigation and direct comparisons between both types of 5-HT_{1A} receptor knockout mice in different benzodiazepine-sensitive paradigms, as well as $GABA_A$ receptor subunit regulation, are in progress.

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