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$GABA_A$–benzodiazepine receptor complex ligands and stress-induced hyperthermia in singly housed mice

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Abstract

Stress-induced hyperthermia (SIH) in singly housed mice, in which the rectal temperature of a mouse is measured twice with a 10-min interval, enables to study the effects of a drug on the basal (T_1) and on the stress-enhanced temperature (T_2), 10 min later, using the rectal procedure as stressor. SIH ($T_2 - T_1$) reflects a stress-induced phenomenon sensitive to stress- or anxiety-modifying effects of drugs. Several benzodiazepine agonists (diazepam, chlordiazepoxide, oxazepam and alprazolam) dose-dependently antagonized SIH either in NMRI mice from two different breeders or in BALB/c mice. No major differences in the sensitivity for any of the drugs tested were found between strains or between substrains from different breeders. The selective BZ_1 receptor agonists alpidem and zolpidem only at relatively high doses antagonized SIH, whereas flumazenil, FG7142, pentylenetetrazol and phenobarbital did not affect SIH. Alcohol antagonized SIH, and the effects of diazepam could be antagonized by flumazenil. The findings that full BZ receptor agonists have anxiolytic-like effects in the singly housed SIH paradigm are comparable to those previously found in the group-housed version. The singly housed SIH is proposed as a simple and reliable screen for detecting anxiety-like properties of drugs that is valid in every mouse strain tested so far.

Keywords: Stress-induced hyperthermia; Mouse; Temperature; Strain; $GABA_A$–benzodiazepine receptor; Anxiety

1. Introduction

Stress-induced hyperthermia (SIH) in singly housed male mice appears to be a robust, reproducible and easy paradigm to study putative anxiolytic-like effects of drugs (Van der Heyden et al., 1997; Olivier et al., 1998). In this paradigm, singly housed mice are subject to two sequential rectal temperature measurements with a 10-min interval. The first measurement is the basal temperature (T_1), the second is the stress-enhanced temperature (T_2) and the difference (ΔT) is the SIH. In a telemetric study (Bouwknecht et al., 2000), the exact course of the core body temperature was followed over the whole experimental period. The basal temperature was increased by the (vehicle) injection procedure, but returned to baseline after approximately 45–60 min. The subsequent rectal temperature measurement procedure led to an increase in body temperature of 1–1.5 °C over the ensuing 10–15 min and waned afterwards. The nontelemetric SIH procedure therefore very adequately measures the basal temperature (T_1) 60 min after the injection and the stress-induced temperature (T_2) 10 min later.

The $GABA_A$–benzodiazepine receptor agonist diazepam and the $5\text{-}HT_{1A}$ receptor agonist flesinoxan both reduced ΔT (Van der Heyden et al., 1997; Olivier et al., 1998; Pattij et al., 2001), reflecting their anxiolytic-like effects. In contrast, the tricyclic antidepressant amitriptyline had no effect (Van der Heyden et al., 1997), suggesting that the paradigm reflects anxiolytic-like specificity.

In a closely related paradigm, SIH in group-housed mice (Zethof et al., 1994) and a broad variety of drugs (Zethof et al., 1995) including benzodiazepines, $5\text{-}HT_{1A}$ receptor

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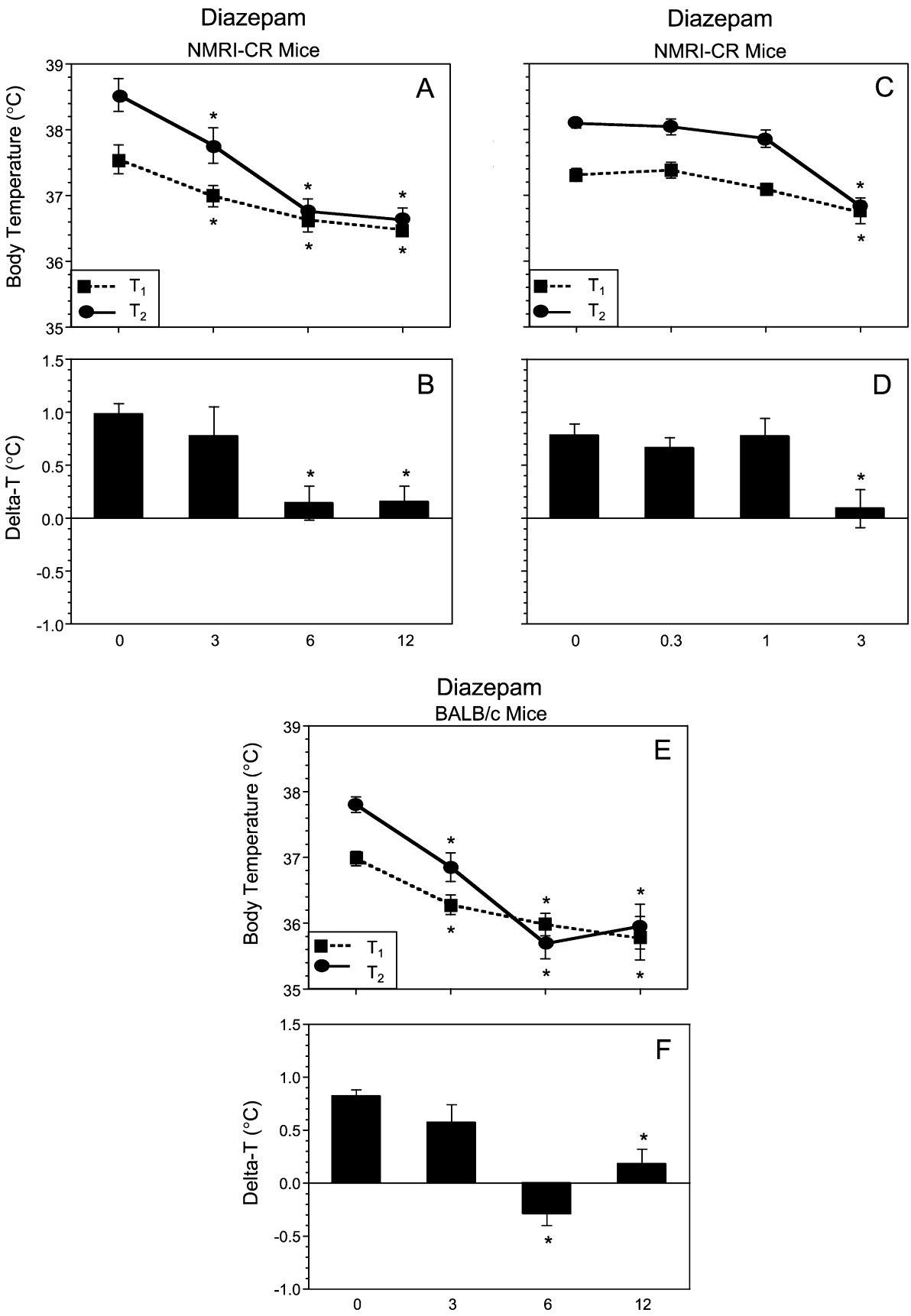
Diazepam
NMRI-CR Mice
A
B
C
D
Body Temperature (°C)
Delta-T (°C)
T1
T2
Diazepam
BALB/c Mice
E
F

agonists, various antidepressants and other drugs were explored showing that this experimental procedure is capable of detecting anxiolytic-like activity of psychoactive drugs. However, the number of mice needed to perform the group-housed SIH is extremely high and a closely related paradigm in singly housed animals was developed (Van der Heyden et al., 1997), using only 10% of the original number of animals.

In the present contribution, the effects of several compounds with differential activity at the $GABA_A$–BZ receptor complex are described in this singly housed SIH paradigm to further investigate its use as a putative animal screen for anxiolytic-like activity. Moreover, some selected drugs in different strains or a similar strain (NMRI) from a different breeder were tested. Such data pertain to the generalization of the SIH paradigm.

2. Methods

2.1. Animals

Male NMRI mice (Charles River, Sulzfeld, Germany) weighing approximately 12–14 g upon arrival were housed in groups of 10 per cage ($34 \times 22 \times 15$ cm) under non-reversed 12-h light–12-h dark cycle conditions (lights on from 07:00 to 19:00 h). For some experiments, mice of another strain (BALB/c) or NMRI mice from another breeder (Harlan, Zeist, The Netherlands) were used. They were housed and treated identically to the NMRI mice. The day before an experiment, mice were individually housed in smaller cages ($12 \times 18 \times 13$ cm). Animals were housed at constant room temperature (21 ± 2 °C) and relative humidity ($60 \pm 10\%$) with food and water freely available. Experiments were carried out in laboratory-adapted animals between 09:00 and 15:00 h at least 1 week after their arrival. After testing, the same mice were regrouped into a cage ($n = 10$). For each experiment per dose group, minimally eight animals were used. All experiments were approved by the IACUC (DEC) from Solvay Pharmaceuticals, Weesp, The Netherlands. All procedures are in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.2. General procedure

Cages were randomly and evenly allocated over daytimes (morning–afternoon) and treatments in each experiment. The temperature of mice was measured by rectally inserting a thermistor probe by a length of 2 cm. Digital temperature recordings were obtained with an accuracy of 0.1 °C using a Keithley 871A digital thermometer (NiCr–NiAl thermocouple). The probe, dipped into silicon oil before inserting, was held in the rectum till a stable rectal temperature had been obtained for 20 s. Animals were injected orally or subcutaneously with either drug or vehicle (tragacanth suspension, 1% w/v), 60 min before the first temperature measurement (T_1). The temperature was again measured 10 min later (T_2). In antagonism studies, the antagonist and agonist were injected 60 min before the first rectal temperature measurement, immediately following each other.

2.3. Drugs

Diazepam-base, oxazepam-base, chlordiazepoxide-base, pentylenetetrazol-base (OPG, The Netherlands), alprazolam-base (Upjohn, USA), alpidem-base (Synthelabo Recherche, France), bretazenil-base, flumazenil-base (Hoffmann La Roche, Basel), phenobarbital-base (Brocacef, The Netherlands) and *N*′-methyl-β-carbolin-3-carboxamide-base (FG7142; RBI, USA) were suspended in a mixture of gelatin (0.5%) and mannitol (5%) in water. Zolpidem 1/2 tartrate (Synthelabo Recherche, Paris) was dissolved in water. All solutions/suspensions were adjusted (if needed) to pH between 5 and 8. Control groups received the appropriate vehicle treatment. All injections were administered orally or subcutaneously in a volume of 10 ml/kg bodyweight.

2.4. Statistics

For each individual mouse, a basal temperature (T_1), an end temperature (T_2) and the difference (ΔT) $= T_2 - T_1$ was determined. After homogeneity of variance for the variables T_1, T_2 and ΔT was checked between treatments, treatment effects were evaluated using a two-way analysis of variance, with explanatory factors treatment and daytime, together with all the interactions. The factor block, containing each dose once, was also implemented in the analysis, but was not significant in any experiment (not presented). If the overall analysis of variance appeared significant, post hoc *t* tests were used to identify significant differences.

3. Results

Although ΔT varied over all the experiments performed, in all cases, a significant SIH (after vehicle injections) was found, ranging from 0.6 to1.0 °C.

Fig. 1. The effects of vehicle or various doses of diazepam in NMRI-CR (top left), NMRI-H (top right) and BALB/c (bottom) mice on the rectal temperature (top part of each panel) are shown ($n = 8$ mice/dose). The vehicle or dose (mg/kg, po) was administered 60 min before the first rectal temperature measurement (T_1), which was followed by a second rectal temperature measurement 10 min later (T_2). The lower part of each panel shows ΔT, the difference between T_2 and T_1. NMRI mice from two different breeders (Charles River and Harlan) were used. Each point is the mean ± S.E.M. * Indicates a significant difference ($P < .05$) from vehicle.

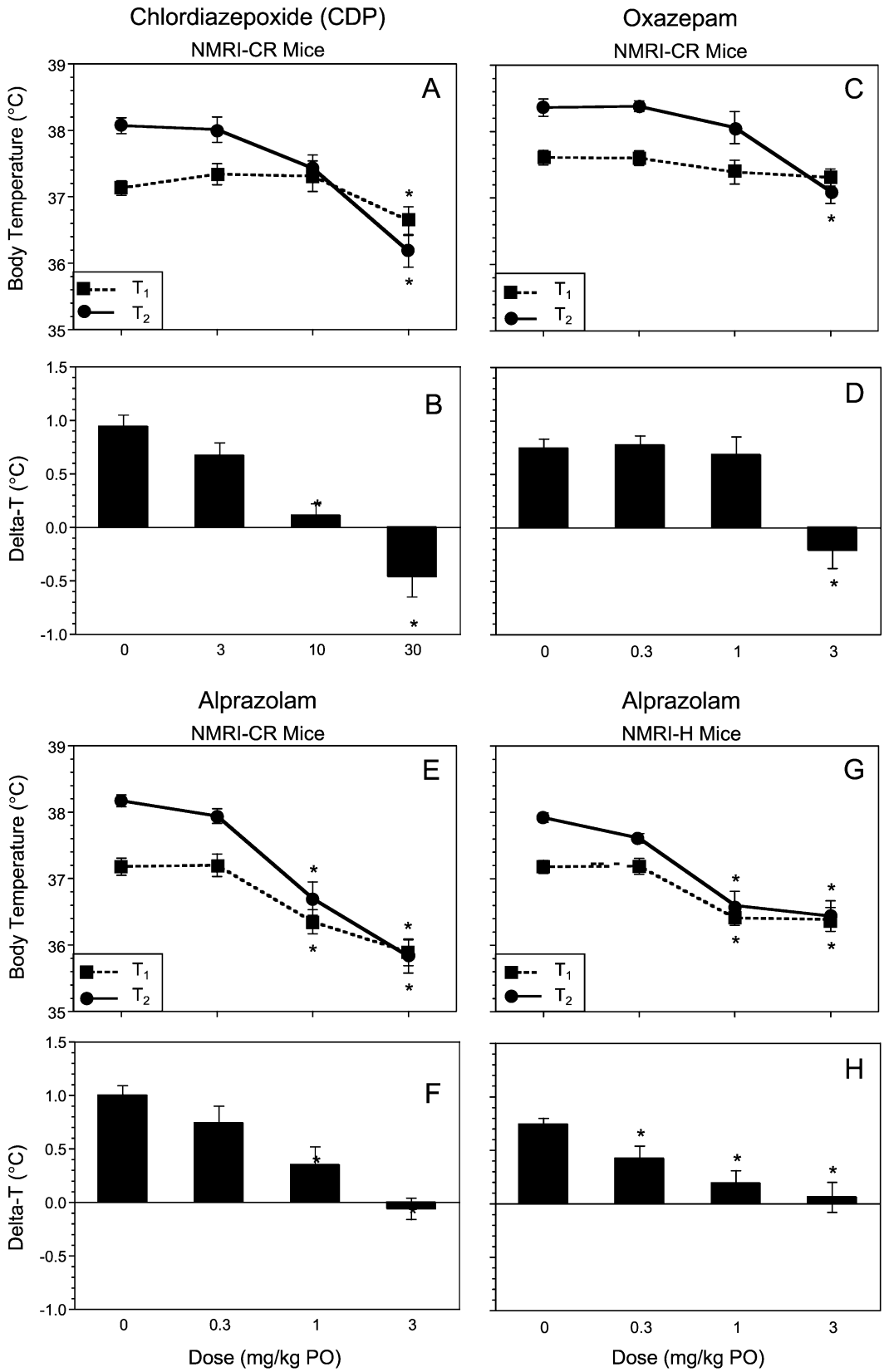

Fig. 2. The effects of vehicle or various doses of chlordiazepoxide (top left), oxazepam (top right) and alprazolam (bottom panels) on the rectal temperature of NMRI mice are shown ($n = 8$ mice/dose, except for the vehicle groups: $n = 16$). Drugs or vehicle were given orally 60 min before the first rectal temperature measurement (T_1), which was followed by a second rectal measurement 10 min later (T_2). The lower panel of each drug study shows ΔT ($T_2 - T_1$). Alprazolam was tested in NMRI mice from two different breeders (Charles River and Harlan). Each point is the mean ± S.E.M. * Indicates a significant difference ($P < .05$) from vehicle.

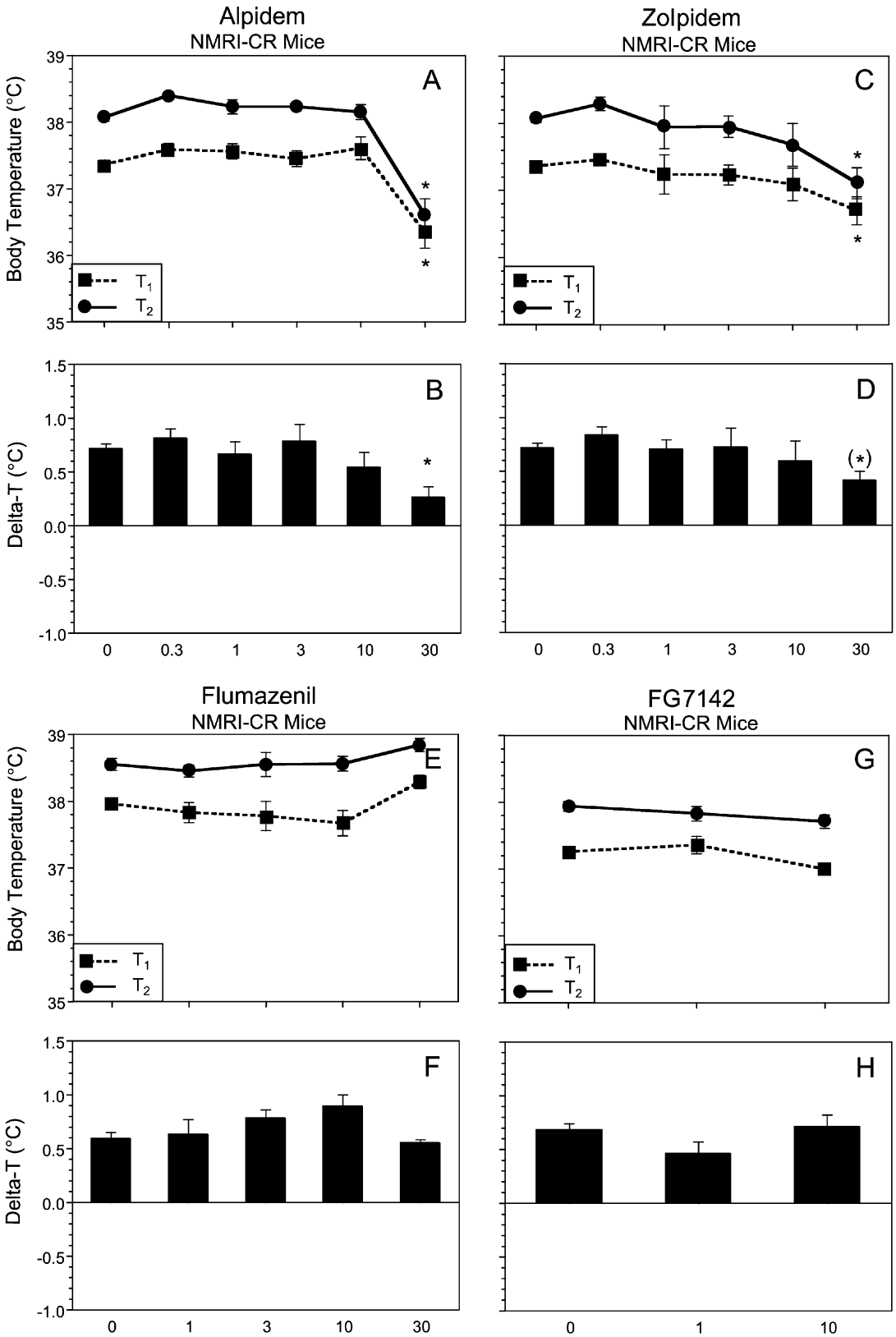

Fig. 3. The effects of vehicle or various doses of alpidem (top left), zolpidem (top right), flumazenil (bottom left) and FG7142 (bottom right) on the rectal temperature of NMRI-CR mice are shown ($n = 8$ mice/dose, except for the vehicle groups of the alpidem and zolpidem experiments: $n = 33$, and the vehicle groups of the flumazenil and FG7142 experiments: $n = 16$). Drugs or vehicle were given orally 60 min before the first rectal temperature measurement (T_1), which was followed by a second rectal temperature measurement 10 min later (T_2). The lower panel of each drug study shows ΔT ($T_2 - T_1$). Each point is the mean ± S.E.M. * Indicates a significant ($P < .05$) difference from vehicle.

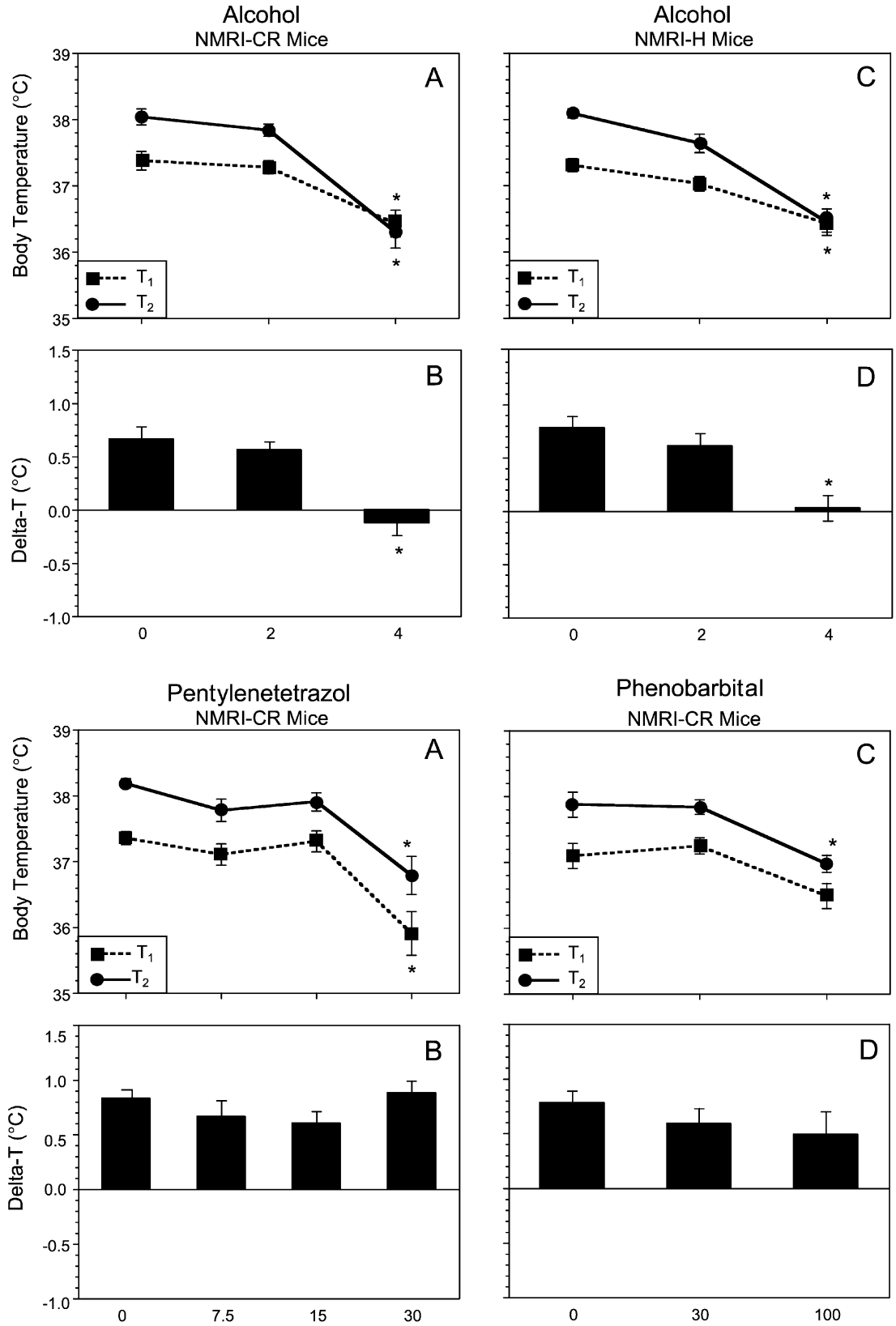

Fig. 4. The effects of vehicle or various doses of alcohol (g/kg) (top panels), pentylenetetrazol (bottom left) and phenobarbital (bottom right) on the rectal body temperature of NMRI mice ($n = 8$ mice/dose, except for the vehicle dose: $n = 16$) are shown. The first rectal temperature measurement (T_1) was taken 60 min after the oral administration of vehicle or a dose of alcohol, followed 10 min later by a second rectal temperature measurement (T_2). In the bottom panel, ΔT ($T_2 - T_1$) is shown. The data are shown for alcohol on NMRI mice from two different breeders (Charles River and Harlan). Each point is the mean ± S.E.M. * Indicates a significant difference ($P < .05$) from vehicle.

Diazepam was tested in NMRI mice derived from Charles River (NMRI-CR) and Harlan (NMRI-H) and in BALB/c mice derived from Harlan (Fig. 1). Diazepam was tested in NMRI-CR mice in a dose range of 3–12 mg/kg po. Analysis of variance showed significant overall effects of diazepam in NMRI-CR mice (Fig. 1, left top panel) on T_1 [$F(3,21)=6.87$; $P<.05$], T_2 [$F(3,21)=15.19$; $P<.0001$] and on ΔT [$F(3,21)=6.0$; $P<.05$]. Post hoc comparisons showed that from 3 mg/kg on, diazepam significantly decreased T_1 and T_2, and from 6 mg/kg on, ΔT. In NMRI-H mice (Fig. 1, right top panel), a significant overall effect was found on T_1 [$F(3,29)=4.20$; $P<.05$], T_2 [$F(3,29)=27.77$; $P<.0001$] and ΔT [$F(3,29)=4.98$; $P<.01$]. Only the highest dose used (3 mg/kg, po) affected all three parameters. In BALB/c mice (Fig. 1, bottom panel), significant overall effects were found on T_1 [$F(3,19)=8.88$; $P<.001$], on T_2 [$F(3,19)=18.59$; $P<.0001$] and on ΔT [$F(3,19)=21.95$; $P<.0001$]. From 3 mg/kg po (and higher), T_1 and T_2 were significantly decreased; ΔT was significantly decreased at 6 and 12 mg/kg po.

Fig. 2 (top left) shows the effects of chlordiazepoxide (0, 3, 10 and 30 mg/kg, po) in NMRI-CR mice. Overall effects were found on T_1 [$F(3,28)=4.52$; $P<.01$], on T_2 [$F(3,28)=31.82$; $P<.0001$] and on ΔT [$F(3,28)=34.59$; $P<.0001$]: T_1 and T_2 were significantly decreased at 30 mg/kg and ΔT at 10 and 30 mg/kg.

Oxazepam in NMRI-CR mice (Fig. 2, top right) had significant overall effects on T_2 [$F(3,29)=11.54$; $P<.0001$] and on ΔT [$F(3.29)=10.54$; $P<.0001$], but not on T_1. At 3 mg/kg, both T_2 and ΔT were significantly decreased.

Alprazolam in NMRI-CR mice (Fig. 2, bottom left) showed significant overall effects on T_1 [$F(3,29)=18.24$; $P<.0001$], T_2 [$F(3,29)=53.53$; $P<.0001$] and ΔT [$F(3,29)=12.83$; $P<.0001$]. T_1, T_2 and ΔT were significantly decreased at 1 and 3 mg/kg po. In NMRI-H mice, alprazolam (Fig. 2, bottom right) had significant overall effects on T_1 [$F(3,28)=12.52$; $P<.0001$], T_2 [$F(3,28)=33.24$; $P<.0001$] and ΔT [$F(3,28)=6.25$; $P<.002$]. T_1 and T_2 were significantly decreased at 1 and 3 mg/kg, whereas ΔT was significantly decreased from 0.3 mg/kg on.

Alpidem (Fig. 3, top left) significantly affected T_1 [$F(5,52)=8.93$; $P<.0001$], T_2 [$F(5,52)=21.65$; $P<.0001$] and ΔT [$F(5,52)=2.69$; $P<.05$]. Only at 30 mg/kg po were all three parameters significantly decreased.

Zolpidem (Fig. 3, top right) had no significant overall effects on T_1 and ΔT but had a significant overall effect on T_2 [$F(5,52)=3.37$; $P<.01$]. T_2 was significantly decreased at 30 mg/kg.

Bretazenil (1, 3, 10 and 30 mg/kg, po) had no effects on T_1 and ΔT (data not shown) but had an overall effect on T_2 [$F(5,52)=3.40$; $P<.01$] and reduced T_2 at 30 mg/kg po.

Flumazenil (Fig. 3, bottom left) and FG7142 (Fig. 3, bottom right) did not affect any of the parameters measured.

Alcohol in NMRI-CR mice (Fig. 4, top left) showed significant overall effects on T_1 [$F(2,22)=10.47$; $P<.001$], T_2 [$F(2,22)=36.85$; $P<.0001$] and ΔT [$F(2,22)=8.97$; $P<.001$]. Alcohol significantly decreased T_1, T_2 and ΔT at 4 g/kg po. Alcohol in NMRI-H mice (Fig. 4; top right) had significant overall effects on T_1 [$F(2,21)=13.19$; $P<.0001$], T_2 [$F(2,21)=57.54$; $P<.0001$] and ΔT

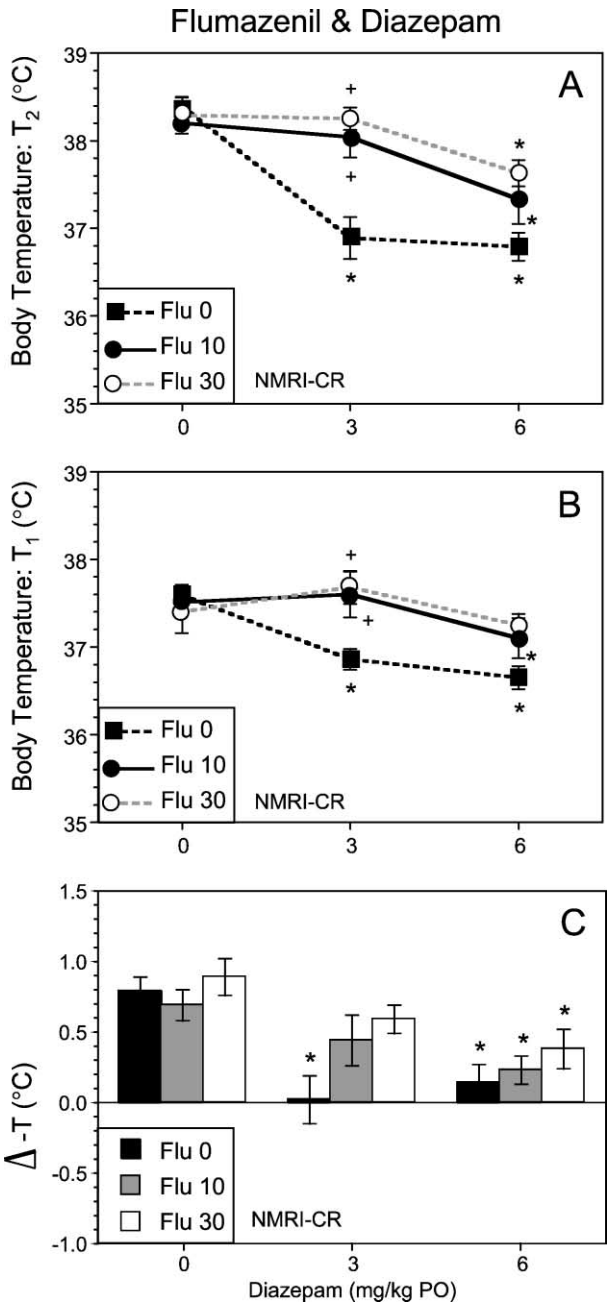

Fig. 5. The effects of the interaction of diazepam (vehicle, 3 or 6 mg/kg, po) with flumazenil (vehicle, 10 or 30 mg/kg, po) are shown on the rectal temperature of NMRI-CR mice ($n=8$ mice/dose, except for the vehicle–vehicle groups: $n=16$). The antagonist (flumazenil) and the agonist (diazepam were simultaneously administered 60 min before the first rectal temperature measurement (T_1; middle panel), followed 10 min later by a second rectal temperature measurement (T_2; top panel). The bottom panel shows ΔT (T_2-T_1). Each point is the mean ± S.E.M. * and + indicate significant ($P<.05$) differences between the vehicle of the same group and the corresponding dose of the vehicle + diazepam group, respectively.

[$F(2,21) = 15.86$; $P < .0001$]. Again, alcohol significantly decreased T_1, T_2 and ΔT at 4 g/kg po.

Pentylenetetrazol (Fig. 4, left bottom panel) had overall significant effects on T_1 [$F(3,29) = 11.53$; $P < .0001$] and T_2 [$F(3,29) = 14.18$; $P < .0001$] but not on ΔT. Only at 30 mg/kg sc did pentylenetetrazol significantly reduce T_1 and T_2, but not ΔT.

Phenobarbital (Fig. 4, right bottom panel) did significantly affect T_1 [$F(2,22) = 4.03$; $P < .05$] and T_2 [$F(2,22) = 6.09$; $P < .01$] but not ΔT. Phenobarbital only decreased T_2 at 100 mg/kg po.

In Fig. 5, the interaction between two doses (3 and 6 mg/kg, po) of diazepam, in combination with vehicle or 10 and 30 mg/kg po flumazenil, is shown. Flumazenil itself, like in the earlier experiment, had no effects either on T_1, T_2 or ΔT. Flumazenil significantly antagonized the effects of diazepam on T_1 [$F(4,62) = 3.32$; $P = .016$], on T_2 [$F(4,62) = 6.25$; $P < .0001$] and on ΔT [$F(4,62) = 14.59$; $P < .0001$]. Both doses of flumazenil antagonized the decreases in T_1, T_2 and ΔT induced by 3 mg/kg diazepam. The vehicle + 6 mg/kg diazepam-induced decrease in T_1, T_2 and ΔT could not be antagonized by either 10 or 30 mg/kg flumazenil.

4. Discussion

Anxiolytic-like effects of a drug in the SIH paradigm in singly housed mice are obtained if ΔT is decreased after drug administration. ΔT is the temperature difference in SIH between two sequentially spaced (10-min interval) rectal temperature measurements in a single animal. This interval was chosen after extensive optimization experiments on the SIH paradigm in singly housed mice (Van der Heyden et al., 1997), and the procedure could be confirmed after extensive telemetric measurements (Bouwknecht et al., 2000). SIH appears independent of the intrinsic effects of a drug on body temperature (T_1). Thus, by injecting a drug 60 min before the first rectal temperature measurement (avoiding the temperature stress induced by the injection; Van der Heyden et al., 1997; Bouwknecht et al., 2000), the effects of a drug on basal body temperature (T_1) and on the stress-induced measure by the rectal procedure (T_2) can be obtained. Operationally, $T_2 - T_1 = \Delta T$ is used as the parameter of stress. When a drug reduces ΔT, the effect is interpreted as antistress or anxiolytic like (Zethof et al., 1995; Van der Heyden et al., 1997; Olivier et al., 1998; Bouwknecht et al., 2000; Pattij et al., 2001). An unchanged ΔT indicates absence of any effect on anxiety or stress, whereas an increased ΔT could indicate an anxiogenic-like effect (cf. Zethof et al., 1995). However, at least in the group-housed version of the SIH in mice, anxiogenic-like effects are difficult, if not impossible to demonstrate, possibly due to a ceiling effect in the end temperature (T_2) (Zethof et al., 1995). This feature weakens the applicability of the SIH as a screen for measuring effects of drugs on anxiety.

The attractiveness of the present SIH paradigm is, besides the 90% reduction in animal number compared to the group-housed version (Van der Heyden et al., 1997), the ease of the procedure. With an injection-test interval of 60–70 min, for most drugs, an acceptable brain concentration can be obtained, both after intraperitoneal, oral or subcutaneous routes. Only for drugs with an "extremely" short duration of action (e.g. 8-OH-DPAT) can the injection-test interval lead to erroneously high doses to detect effects. However, a shorter injection-test interval leads to an already enhanced body temperature at the first rectal measurement (T_1), which interferes with the outcome of the experiment (Bouwknecht et al., 2000; Van der Heyden et al., 1997).

Under these conditions, several compounds with a variety of actions at the $GABA_A$–BZ receptor complex were examined. BZ receptor agonists are reputed for their anxiolytic-like actions, and we hypothesized that such drugs will exert anxiolytic-like activity in the SIH, like in the group-housed version (Zethof et al., 1995).

The full BZ receptor agonists diazepam, oxazepam, chlordiazepoxide and alprazolam, which are not selective for specific subunits of the $GABA_A$–BZ receptor complex, decreased ΔT in the standard mouse strain used, the NMRI (Charles River), indicative of a strong anxiolytic-like effect. Interestingly, the decreases in ΔT were independent from basal effects of the drug on temperature (T_1). Diazepam (max -1.07 °C), alprazolam (max -1.29 °C), chlordiazepoxide (max -0.49 °C) and oxazepam (max -0.30 °C; not significantly) all decreased T_1, but reduced T_2 more intensively, even leading to negative ΔT's (alprazolam, oxazepam, diazepam and chlordiazepoxide; $\Delta T_{max} = -0.06$, -0.21, $+0.15$ and -0.46 °C, respectively). This once more illustrates the power of the SIH procedure in which, independent of sensory, motor or intrinsic effects of drugs on temperature, the SIH can be measured. Anxiolytic-like effects of nonsubunit selective BZ receptor agonists were also found in the group-housed SIH (Borsini et al., 1989; Lecci et al., 1990; Zethof et al., 1995). The present data on diazepam and alprazolam are highly similar to those in Zethof et al. (1995), performed on the same strain (NMRI-CR) and using the same procedure for administration of drugs. This can be taken as evidence, besides the extreme reduction of the number of animals used, that the single-housed version of the SIH is an excellent alternative for the group-housed version. In order to generalize the SIH method, a number of drugs were tested, including diazepam, alprazolam and alcohol in two different strains (NMRI and BALB/c), but also in the same strain (NMRI) from different breeders (Charles River and Harlan). NMRI mice from different suppliers did not differ dramatically in their sensitivity towards diazepam in the SIH procedure (Fig. 1). Although only tested up to 3 mg/kg, NMRI-H mice showed effects of diazepam comparable to NMRI-CR and BALB/c mice. Basically, all three types of mice showed a comparable effect on the rectal stressor, a ΔT of approximately 0.9–1.0 °C. Alprazolam and alcohol had quite comparable pro-

files in NMRI-CR and NMRI-H, indicating that NMRI-H mice from different breeders seem to be overall equally sensitive to effects of drugs. Recently, SIH was also reliably found in a number of 129 Sv strains (Bouwknecht et al., 2000; Pattij et al., 2001). These data support the notion that SIH is a very general phenomenon in every mouse strain tested so far and seems generally applicable.

The relatively selective partial $BZ_{1(\omega1)}$ receptor agonist alpidem (Langer et al., 1990) had anxiolytic-like activity in the SIH, although only at a relatively high dose. The selective, full $BZ_{1(\omega1)}$ receptor agonist zolpidem (Langtry and Benfield, 1990; Sanger et al., 1987) had a comparable profile; it had marginal anxiolytic-like activity at a relatively high dose (30 mg/kg). The pharmacologically defined BZ_1 and BZ_2 sites (Squires et al., 1979) correspond with affinity of compounds for different subunit composition of the $GABA_A$–BZ receptor. BZ_1 (mainly of $\alpha_1\beta_2\gamma_2$ subunit composition) and BZ_2 sites (mainly of α_2/α_3, β_x and γ_2 subunit composition) are equally sensitive to the classic benzodiazepines but differ in their affinity for compounds like alpidem and zolpidem, which have selective affinity for BZ_1 sites, but no affinity for BZ_2 sites (Möhler et al., 2000; Whiting et al., 2000). There is considerable evidence that, in particular, the α_2 subunit of the $GABA_A$–BZ complex is involved in the modulation of anxiety (Löw et al., 2000; Rudolph et al., 1999), whereas the α_1 subunit seems to be involved primarily in sedation (McKernan et al., 2000). This nicely fits with the present findings that alpidem and zolpidem are not, or only marginally and at high doses, anxiolytic-like in the SIH. Alpidem showed anxiolytic-like activity in some animal models, such as marble burying, punished drinking (Vogel test), feeding under stress (Zivkovic et al., 1990), elevated plus maze (Sanger et al., 1994) and ultrasonic vocalizations in rat pups under mild stress conditions (Olivier et al., 1998). On the other hand, alpidem was not anxiolytic in other anxiety paradigms, such as shock-induced fighting, four-plate test, Geller–Seifter conflict procedure (Zivkovic et al., 1990) or the high-stress condition of the rat pup ultrasonic vocalizations (Olivier et al., 1998). Zolpidem, a full BZ_1 receptor agonist, selective for α_1 subunits in the $GABA_A$–BZ receptor complex and a clinically useful hypnotic (Langtry and Benfield, 1990; Sanger et al., 1987), had shown some anxiolytic-like effects, e.g. in the water-lick conflict test (Depoortere et al., 1986). However, it was mainly inactive in classical anxiolytic screens in which nonselective BZ receptor agonists were very efficacious (Depoortere et al., 1986). In contrast, zolpidem was very active and efficacious in the rat pup ultrasonic vocalization test, both under low- and high-stress conditions (Olivier et al., 1998). Remarkably, bretazenil, a partial benzodiazepine receptor agonist, was, up to a dose of 30–mg/kg, not active in SIH, although because of the lowering effect on T_2 at 30 mg/kg, it could have anxiolytic-like activity at higher doses. However, bretazenil has high affinity for α_4 and α_6 subunits (Whiting et al., 2000), which have no affinity for classical benzodiazepine receptor agonists like diazepam and are not involved in anxiety processes (Whiting et al., 2000). Alcohol, putatively interacting with some allosteric site at the $GABA_A$–BZ receptor complex (Rabow et al., 1995), clearly showed anxiolytic-like activity, although at a relatively high dose. In the group-housed SIH, a comparable activity profile of alcohol was found (Zethof et al., 1995). Phenobarbital, acting through a putative barbiturate site at the $GABA_A$–BZ receptor complex, of which the subunit specificity is not yet known (Sieghart, 1995; Rabow et al., 1995; Whiting et al., 2000), had, up to an oral dose of 100 mg/kg, no anxiolytic-like activity. In the group-housed SIH, phenobarbital was tested up to 60 mg/kg, but via the intraperitoneal route, and had also no effects (Zethof et al., 1995). The high dose (100 mg/kg, po) used here clearly had hypnotic activity in the mice tested (visual observation), however, although temperature (T_1 and T_2) overall decreased, no effects on ΔT were found. Our findings contrast those of Lecci et al. (1990) in the SIH procedure where anxiolytic-like activity was found at a relatively low dose (20 mg/kg, ip). This discrepancy could be due to strain differences. Pentylenetetrazol, acting via the picrotoxin site at the $GABA_A$–BZ receptor complex (Rabow et al., 1995) had no effect on ΔT, although it decreases T_1 and T_2 at the highest dose (30 mg/kg, sc) tested. Pentylenetetrazol was capable of inducing convulsions at high doses and was found anxiogenic like at lower doses (Rodin and Calhoun, 1970; Stephens and Andrews, 1991). In the group-housed version (Zethof et al., 1995) even anxiolytic-like activity at 30 mg/kg sc was found. The β-carboline, FG7142, a partial inverse receptor agonist at the $GABA_A$-BZ receptor complex, with anxiogenic-like properties in man (Dorow et al., 1983) and animals (Cole et al., 1995), had no effect in our procedure. Although higher doses and different routes of administration should be tested, the findings were in line with the findings after pentylenetetrazol. Apparently, the SIH paradigm in mice, independent whether singly housed or group-housed, fails to detect anxiogenic effects of drugs (e.g. pentylenetetrazol, *m*-CPP and FG7142). This weakens the SIH procedure considerably as a screen to detect effects of drugs on anxiety. The BZ receptor antagonist flumazenil had, up to 30 mg/kg po, no intrinsic effects in the SIH procedure. Flumazenil (10 and 30 mg/kg, po) was able to fully antagonize the effects of 3 mg/kg po diazepam and, partly, the effects of 6 mg/kg po diazepam. Probably, higher doses of flumazenil are needed to fully antagonize the latter dose of diazepam. The antagonism of the effects of diazepam on SIH by flumazenil illustrates the involvement of the $GABA_A$–BZ receptor complex in the modulation of the SIH.

In conclusion, the SIH paradigm in singly housed mice is present in different (sub) strains and is sensitive to anxiolytic-like effects of BZ receptor agonists. This procedure does not detect anxiogenic-like effects of drugs. The SIH paradigm is an easy anxiety/stress related screen to detect anxiolytic-like properties of drugs.

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