Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 463 (2003) 117–132
www.elsevier.com/locate/ejphar

Stress-induced hyperthermia and anxiety: pharmacological validation

Berend Olivier[a,b,c,*], Theo Zethof[d], Tommy Pattij[a], Meg van Boogaert[a], Ruud van Oorschot[a,d], Christina Leahy[c], Ronald Oosting[a], Arjan Bouwknecht[a,e], Jan Veening[f], Jan van der Gugten[a], Lucianne Groenink[a]

[a] *Department of Psychopharmacology, Utrecht Institute of Pharmaceutical Sciences, Utrecht University, Sorbonnelaan 16, 3584CA Utrecht, The Netherlands*
[b] *Department of Psychiatry, Yale University School of Medicine, New Haven, CT, USA*
[c] *PsychoGenics Inc., 4 Skyline Drive, Hawthorne, USA*
[d] *Solvay Pharmaceuticals, Weesp, The Netherlands*
[e] *Baylors College of Medicine, Houston, TX, USA*
[f] *Department of Anatomy and Embryology, University of Nijmegen, The Netherlands*

Accepted 10 December 2002

Abstract

When mammals, including man, are confronted with a stressful event, their core body temperature rises, stress-induced hyperthermia. In mice, the stress-induced hyperthermia procedure has been developed to measure antistress or anxiolytic-like effects of psychoactive drugs. Group-housed and singly housed versions of the stress-induced hyperthermia generate comparable results. Because the number of animals needed to perform an experiment is much lower in the singly housed versus the group-housed procedure, the former is the test of choice for pharmacological testing. A typical stress-induced hyperthermia test starts with an injection 60 min before the first rectal temperature measurement (T_1), followed by a second temperature measurement (T_2) 10–15 min later. The difference ΔT ($=T_2-T_1$) is the stress-induced hyperthermia. The procedure also measures the intrinsic activity of drugs on the basal body temperature and ΔT is relatively independent from the intrinsic temperature effects of drugs. Anxiolytic drugs (benzodiazepines, 5-HT_{1A} receptor agonists, alcohol) reduce ΔT suggestive of anxiolytic-like effects. Because the parameter measured for anxiety in the stress-induced hyperthermia procedure is not dependent on locomotor activity, like in almost all other anxiety tests, the stress-induced hyperthermia procedure is an attractive addition to tests in the anxiety field. Because the stress-induced hyperthermia is also present with a comparable pharmacological profile in females, this procedure has a wide species and gender validity. The procedure was applied in various genetically modified mice [5-HT_{1A} and 5-HT_{1B} receptor knockout (KO) mice and corticotropin-releasing hormone overexpressing (CRH-OE) mice] to study phenotypic influences of the various mutations on aspects of anxiety. The stress-induced hyperthermia test in singly housed male and female mice appears a useful and extremely simple test to measure effects of drugs on certain aspects of anxiety or to help to determine phenotypic differences in mutant mice.

Keywords: Stress; Anxiety; Stress-induced hyperthermia; Body temperature; Mutant mouse

1. Introduction

Stress-induced hyperthermia is an integral part of an individual's response to situations perceived as threatening or distressing by that individual (Reeves et al., 1985; Marazziti et al., 1992). Stress-induced hyperthermia is mediated by the autonomic nervous system and occurs both prior to and during exposure to anxiogenic or stress-inducing stimuli, like noise, heat, handling, novelty or pain (Kleitman, 1945; Renbourn, 1960; Bermant et al., 1979; Briese, 1995; Briese and DeQuijada, 1970; Marazziti et al., 1992). In many anxiety disorders, it occurs as an integral part of the pathology and is often considered a representative symptom of the disease, e.g. in generalized anxiety as classified in DSM-IV. The stress-induced hyperthermia test in mice was developed more than a decade ago by the group of Borsini et al. (1989). These authors used group-housed mice (15 mice/cage) and took, every minute, one mouse from the cage and measured its rectal temperature. The last mouse in the cage displayed a higher body temperature than

* Corresponding author. Department of Psychopharmacology, Faculty of Pharmaceutical Sciences, Utrecht Institute of Pharmaceutical Sciences, Utrecht University, Sorbonnelaan 16, 3584CA Utrecht, The Netherlands. Tel.: +31-30-253-3529; fax: +31-30-253-7900.
E-mail address: b.olivier@pharm.uu.nl (B. Olivier).

0014-2999/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0014-2999(03)01326-8

the first mouse and this stress-induced hyperthermia was explained as caused by anticipatory anxiety. In a later developed test, stress-induced hyperthermia in singly housed male mice (Van der Heyden et al., 1997), the rectal temperature of one mouse was measured twice with an interval of 10 min. The difference between these two measurements (ΔT) is the stress-induced hyperthermia and varies between 0.5 and 1.5 °C, depending on strain and time of the day (Peloso et al., 2002). In the group-housed stress-induced hyperthermia versions used by Borsini's group (15 mice/cage) and a modified version (Zethof et al., 1994) that used 10 mice/cage, extensive pharmacological profiling was performed, suggesting that the group-housed stress-induced hyperthermia paradigm was a reliable screen to detect putative anxiolytic-like properties of psychoactive drugs. Moreover, because the temperature of the first mouse measured reflects the basal core temperature of the animal, effects of drugs on this basal body temperature could be measured in the same procedure. The pharmacological data gathered in these various procedures indicated that the stress-induced hyperthermia occurred independently from effects of drugs on the basal temperature. A weak point in the procedure appeared the impossibility to detect anxiogenic effects of drugs (Zethof et al., 1995). The pharmacology performed in the singly housed stress-induced hyperthermia paradigm showed an extremely high similarity to that of the group-housed version (Van der Heyden et al., 1997; Olivier et al., 1998, 2002; Spooren et al., 2000, 2002). This finding, together with the strongly reduced number of mice (only 10%) needed to perform the singly housed test compared to the group-housed stress-induced hyperthermia test, has made the group-housed test almost obsolete. Moreover, physiological and behavioral studies on the time course of stress-induced hyperthermia have shown that both the group-housed and the singly housed versions lead to a similar distribution of the enhanced temperature over time. In group-housed stress-induced hyperthermia (Fig. 1A), temperature rose rapidly after sequential measurements of the individual 10 mice in a cage, being maximally around 8–10 min and waning after 20–30 min, and returning to baseline after about 45–60 min. In the singly housed stress-induced hyperthermia, where individually housed animals are measured twice with varying intervals between T_1 and T_2, a similar time course of stress-induced hyperthermia was found (Fig. 1B).

In order to optimise the stress-induced hyperthermia procedure, we applied it to animals equipped with telemetric devices that measured core body temperature, heart rate and activity (Bouwknecht et al., 2000). Fig. 1C shows the time course of core body temperature measured telemetrically, indicating a similar time course as either via the group-housed or singly housed stress-induced hyperthermia hand-measured version. It clearly can be seen that the injection procedure (handling + injection) is also a considerable stressor in which it evokes a similar increase in core body temperature as the rectal temperature measurement procedure. This stress effect has waned after 45–60 min and the basal body temperature returned to undisturbed baseline. The stress-induced hyperthermia upon the rectal procedure clearly shows that the interval of approximately 10–15 min is very adequate to measure the maximal stress response and

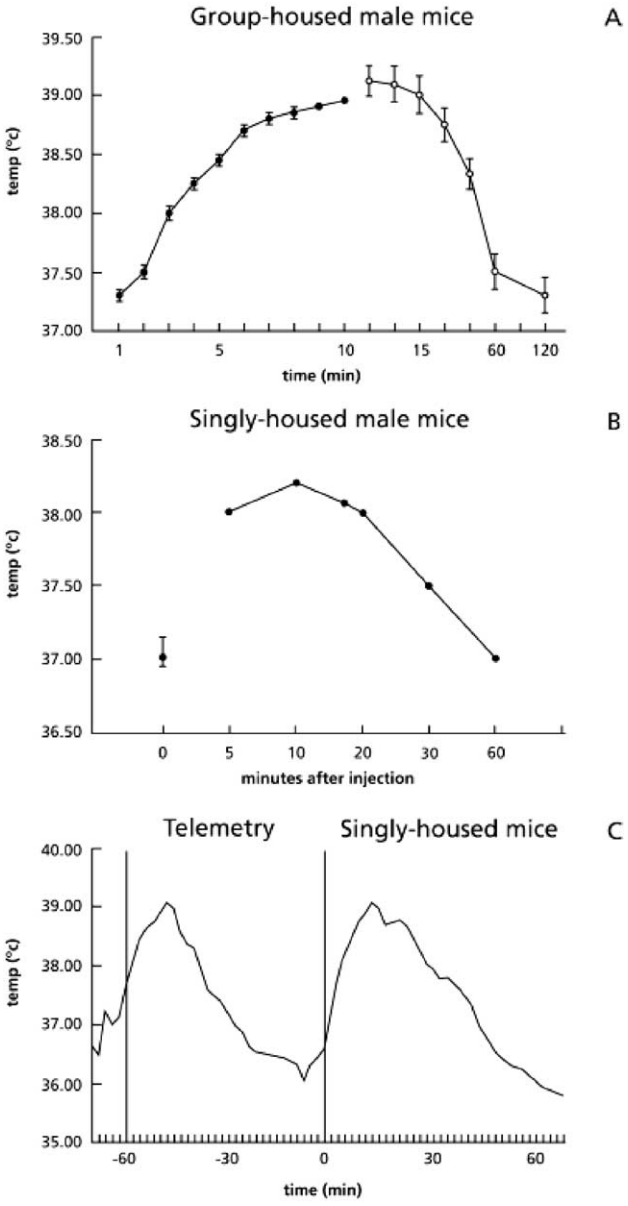

Fig. 1. (A) Course of body temperature in group-housed ($N = 10$ mice/cage: 10 cages) male NMRI mice. Mice were taken with 1-min intervals from the cage and their rectal temperature was measured (1–10 min). For the other time intervals, animals from the same cages were measured again after a certain time interval. (B) The rectal temperature of singly housed male mice of the NMRI strain was measured twice with different time intervals reflecting the duration of the rise in body temperature caused by the rectal procedure stressor. (C) The course of the core body temperature of a male 129SvEv mouse equipped with a telemetric device to record body temperature. The injection of saline enhances body temperature for approximately 45–60 min. The rectal temperature procedure induces another rise in temperature (stress-induced hyperthermia), waning again after 45–60 min.

also yields the optimal period to determine drug effects on stress-induced hyperthermia.

In all inbred and outbred strains measured so far, including NMRI, C57BL/6J, DBA/2J, 129SvEv, 129Sv/Ola, Balbc and Swiss-Webster mice, a clear stress-induced hyperthermia has been found (Bouwknecht and Paylor, 2002). Although differences occur between strains in the basal core temperature (T_1) and the stress-induced enhanced temperature (T_2), and consequently in ΔT, it is yet unknown whether genetic differences underlie these differences, or that experimental factors also play a role, like time of the day or year, testing in the light or dark phase of the light–dark cycle, age of animals, exact housing conditions and gender (no female mice have been studied so far). Thus far, stress-induced hyperthermia has been produced in every strain of mouse tested showing its very robust character.

2. Stress-induced hyperthermia in group-housed mice: pharmacology

When an animal test is proposed to model an affective process, in this case anxiety, such a paradigm should ideally have face, construct and predictive validity (Willner, 1991). Stress-induced hyperthermia seems to have high face validity in which a stress-induced temperature rise appears a universal phenomenon occurring in many species, e.g. in rat (Briese and DeQuijada, 1970; Briese and Cabanac, 1991), in rabbit (Yokoi, 1966) and man (Marazziti et al., 1992; Yoshiue et al., 1989; Yazumi et al., 1989). A model has construct validity if the mechanism behind the pathology seems to have similar physiological substrates in the animal model and in man. Marazziti et al. (1992) found strong support that stress-related hyperthermia in man uses similar physiological and endocrinological mechanisms as those shown in animals. Although temperature regulation is an integrative response to different stimuli at the level of the hypothalamus, it is tempting to hypothesize that, both in humans and in animals, similar mechanisms are involved, including corticotropin-releasing hormone (CRH). CRH is involved in the regulation of a variety of responses occurring in stress and anxiety.

The predictive validity has to be established by testing psychoactive drugs, which are clinically either active or inactive in anxiety disorders and which should have a comparable (in)efficacy in the animal model under study. The data presented here support this.

In our procedure, stress-induced hyperthermia (ΔT) is calculated as the difference between the basal T_1 (mouse 1) and the end T_2 (mouse 10) of group-housed male mice. Drugs can affect the basal temperature and/or end temperature and several interactions may occur, all having consequences for the conclusions about effects of drugs on stress-induced hyperthermia. The stress-induced rise in rectal temperature can be blocked by various mechanisms.

A number of theoretical outcomes of drug effects on T_1 (mouse 1) and on T_2 (mouse 10) are possible. If a drug does not affect T_1, this drug can affect ΔT in three ways: either no influence (no anxiolytic activity), a decrease in ΔT (interpreted as a real anxiolytic-like effect) and an increase in ΔT (interpreted as an anxiogenic-like effect). If a drug induces basically a hypothermic effect, a parallel course of T_1 and T_2 curves indicates absence of any effect, whereas an anxiolytic-like effect is found if the decline in T_2 is steeper than the decline in T_1. If T_2 is not decreased or even enhanced, this could be interpreted as an anxiogenic-like effect. When a drug after temperature enhances the basal temperature can by applied. Several questions occur using this theoretical model. An important one is whether there is a ceiling in the maximal T_2 that can be reached, i.e. can the T_2, which is already 1–2 °C above basal T_1 after vehicle treatment, be further enhanced? Extensive evidence suggests that, indeed, such a ceiling limits the predictability of the model, especially when trying to find anxiogenic-like effects.

As already indicated, anxiolytic-like effects of drugs can be found when the effect on the stress-induced rise in T_2 (mouse 10) is more decreased than the basal T_1 (mouse 1). In a previous study (Zethof et al., 1994), we have extensively investigated the optimal circumstances for drug testing and have found that an injection-test interval of 60 min is necessary to avoid residual effects of the injection procedure on the basal temperature. This has been confirmed telemetrically (Fig. 1C). Using this procedure, it is possible to observe drugs that decrease, increase or have no intrinsic effects on T_1. Similarly, it is possible to detect anxiolytic-like effects of drugs, i.e. the decreasing effect of a drug is stronger on the temperature of mouse 10 than on mouse 1. The predictive validity of a model would improve considerably when it is also sensitive to anxiogenic drugs. The group-housed stress-induced model in mice, however, fails to pick up anxiogenic compounds [e.g. pentylenetetrazol (PTZ), meta-chlorophenylpiperazine (mCPP)]. We have reason to postulate that, under the experimental circumstances, the body temperature of mice cannot be further enhanced than the 1.0–1.5 °C observed. In an extensive range of experiments, it was found that T_2 is more or less fixed, as in all experiments, T_2 of drug-treated mice was maximally 0.1 °C higher compared to T_2 of vehicle-treated mice. This means that an anxiogenic-like effect cannot be deduced from a further enhancement of stress-induced hyperthermia because of a ceiling effect in the maximally reachable temperature. In 29 experiments on male NMRI mice, the average T_1 was 37.89 ± 0.04 °C (range 37.45–38.31 °C) under vehicle conditions, whereas the average T_2 was 39.38 ± 0.03 °C (range 38.86–39.70 °C) under vehicle conditions, with an average ΔT of 1.49 ± 0.04 °C (range 0.97–1.95 °C).

Under drug conditions, increases in T_1 can easily be detected, but increases in T_2 are scarce and, if present, nonsignificant (in the range of 0.1–0.2 °C). The ceiling in T_2 is perhaps best illustrated with D,L-amphetamine. T_1 was, at low doses, dose-dependently decreased from 38.02 °C (0 mg/kg) to 36.92 °C (3 mg/kg) followed by a dramatic increase at 10 mg/kg to 39.28 °C, which is significantly

higher than the basal vehicle temperature. T_2 tends to parallel the basal temperature, except at the 10 mg/kg dose, where the increase seemed dampened by the (physiological?) ceiling. It seems, therefore, that the ceiling effect in T_2 precludes the measurement of anxiogenic-like effects. The groups of Borsini et al. (1989) and Lecci et al. (1990a,b) used a somewhat different procedure to measure stress-induced hyperthermia and postulated that they can observe anxiogenic effects. They exploited groups of 20 mice/cage and found that yohimbine increased the number of hyperthermic mice (8 under vehicle conditions versus 17 mice under yohimbine treatment). In another study (Lecci et al., 1990b), the possible anxiogenic properties of metatrifluoromethylphenylpiperazine (TFMPP, 5 or 20 mg/kg i.p.) could not be detected. Also mCPP, which shows anxiogenic activity in man (Charney et al., 1987) and animals (Kenneth et al., 1989), failed to show an anxiogenic profile in their 20-mice paradigm. In our 10-mice paradigm, we also did not observe an increased number of hyperthermic mice after administration of the putative anxiogenic drugs, PTZ or mCPP. Thus, the detection of anxiogenic-like effects of drugs is difficult, probably due to the more or less fixed maximum reachable temperature. Another implication of the fixed T_2 will be that ΔT can be decreased due to an increased basal T_1. In this case, the decreased ΔT is probably not a real anxiolytic-like effect but a false positive result due to the increased basal T_1 in combination with the fixed end T_2. This implicates that an increased basal T_1 interferes with stress-induced hyperthermia putatively resulting in nonspecific effects.

Therefore, it can be concluded that anxiolytic-like activity of drugs can be easily found in this model, whereas determination of anxiogenic activity is doubtful. Stress-induced hyperthermia could specifically be prevented by prior treatment with drugs possessing anxiolytic activity. The anxiolytic activity of these drugs is not restricted to one mechanism of action. In this model, classic benzodiazepine receptor agonists (diazepam, chlordiazepoxide, alprazolam and others), 5-HT_{1A} receptor agonists (flesinoxan, buspirone, but not ipsapirone) and ethanol are active in blocking stress-induced hyperthermia.

Antidepressants are inactive in blocking stress-induced hyperthermia. The mixed noradrenergic/serotonergic reuptake inhibitors imipramine, amitriptyline, desipramine and clomipramine are inactive in blocking stress-induced hyperthermia, whereas the specific serotonin reuptake inhibitor fluvoxamine partly, not dose-dependently, and nonspecifically antagonized stress-induced hyperthermia due to an increased basal T_1. The serotonin reuptake enhancer tianeptine and the catecholamine reuptake inhibitor nomifensine also nonspecifically antagonized stress-induced hyperthermia due to an increased basal T_1. The 5-HT releaser D,L-fenfluramine also nonspecifically antagonized stress-induced hyperthermia due to an increased basal temperature. These results are consistent with earlier findings (Lecci et al., 1990a,b) and suggest that enhancement of monoaminergic transmission does not play an important role in the antagonism of stress-induced hyperthermia.

Several drugs acting via stimulating or blocking serotonin receptors were also tested in this model (Zethof et al., 1995). Some 5-HT_{1A} receptor agonists (flesinoxan, buspirone) are active in blocking stress-induced hyperthermia, however, with the exception of ipsapirone. It is unclear why the partial receptor agonist ipsapirone was ineffective in blocking stress-induced hyperthermia, whereas buspirone, with a comparable mechanism of action, is active. Perhaps the dopaminergic (D_2) receptor antagonistic properties of buspirone have an additional effect. The anxiolytic-like activity of flesinoxan in this paradigm could be antagonized dose-dependently by 5-HT_{1A} receptor antagonists (unpublished data). The 5-$HT_{2A/2C}$ receptor agonist DOI [1-((2,5)-di-methoxy-4-iodo)-phenyl)-2-amino-propane; Olivier et al., 1993] blocked stress-induced hyperthermia due to a non-dose-dependent increase of basal T_1, also found by Salmi et al. (1994), which we interpreted as a nonspecific effect. The mixed 5-$HT_{1A/1B}$ receptor agonist eltoprazine did not block stress-induced hyperthermia significantly. The 5-$HT_{1B/2C}$ receptor agonist mCPP antagonized stress-induced hyperthermia at the highest dose tested (10 mg/kg p.o.), probably due to the nonsignificant increase in T_1. The 5-$HT_{2A/2C}$ receptor antagonist ketanserine did not block stress-induced hyperthermia, although a large decrease in T_1 was observed. The results of ketanserine showed that stress-induced hyperthermia may occur independently from a decrease in T_1. The 5-HT_3 receptor antagonist ondansetron, which is known to possess anxiolytic activity in some anxiety tests (Olivier et al., 1992, 2000; Costall et al., 1989a,b), was inactive in preventing stress-induced hyperthermia. Our results were generally highly consistent with those of Lecci et al. (1990a,b, 1991) and showed that anxiolytic activity of 5-HT_{1A} receptor agonists but not of 5-HT_2 and 5-HT_3 receptor agonists/antagonists can be detected in this model. The dopamine receptor antagonist haloperidol and the dopamine receptor agonist apomorphine were inactive in blocking stress-induced hyperthermia. These results are consistent with those of Lecci et al. (1990a,b) where it was reported that dopamine D_1/D_2 receptor antagonists did not influence stress-induced hyperthermia, indicating that the dopaminergic system is probably not critically involved in stress-induced hyperthermia. The results with apomorphine again show that stress-induced hyperthermia can operate independently from a decrease in T_1. The antipyrogenic acetylsalicylic acid showed a significant, although limited, increase in T_1 resulting in a non-specifically decreased ΔT. Lecci et al. (1990a) reported a nonsignificant increase in T_1 and also concluded that other mechanisms than those involved in fever are involved in stress-induced hyperthermia.

The catecholamine releaser D,L-amphetamine showed peculiar results as mentioned before. At the dose of 3 mg/kg i.p. T_1 was decreased, while it was increased at 10 mg/kg. However, ΔT was only at 10 mg/kg nonspecifically

decreased. The results of Lecci et al. (1990c) also showed an increase in T_1 at 10 mg/kg, but surprisingly, T_2 was also significantly increased, even above the T_2 of the control mice. Apparently, in the Lecci et al. (1991) study, T_2 was not maximal, whereas our results strongly pointed to a fixed maximal T_2. However, in both stress-induced hyperthermia models, amphetamine did not show specific anxiolytic-like properties.

The antihypertensive drugs clonidine (α_2-adrenoceptor agonist) and prazosine (α_1-adrenoceptor antagonist) did prevent stress-induced hyperthermia but also produced a decreased T_1 at all doses tested. The ΔT was only at the highest dose tested significantly reduced. The results of prazosine were a replication of those obtained by Lecci et al. (1990a) who suggested that the anxiolytic effects of prazosine can be explained by their centrally mediated antihypertensive effects and are not due to their temperature lowering effects. The central CCK_2 receptor antagonist MSD 365260 with potential anxiolytic activity (Singh et al., 1991) did not block stress-induced hyperthermia indicating that this mechanism apparently does not play a role in this model.

The noncompetitive NMDA receptor antagonist dizocilpine (MK 801), which shows anxiolytic activity in some animal models (Dunn et al., 1989), did not block stress-induced hyperthermia at low doses. At the highest dose of 0.3 mg/kg p.o. T_1 was increased significantly. T_2 was increased 0.36 °C above vehicle T_2, so ΔT was not significantly reduced. These results are consistent with findings of Lecci et al. (1991), but they also tested a higher dose of 1 mg/kg p.o. MK 801 and reported an increased T_1 and, surprisingly, a decreased T_2, resulting in a decreased ΔT. We have also tried 1 mg/kg MK 801, but because of convulsions, no data could be obtained.

In conclusion, results in our stress-induced hyperthermia paradigm in male group-housed mice show a more or less fixed maximal end temperature T_2 (physiological limit), which implicates that putative anxiogenic properties of compounds cannot be detected easily in this model. However, anxiolytic properties of drugs can be detected and this is not limited to one mechanism of action. In this model, benzodiazepines, 5-HT_{1A} receptor agonists and alcohol can be found anxiolytic-like. An advantage of this relative simple and robust stress-induced hyperthermia model is the fact that it can be used without disturbances of test compounds on locomotor activity, feeding, drinking and nociception. This model also does not require time-consuming training paradigms, so this model has certain benefits for detecting axiolytic drugs.

3. Endocrinology

Exposure of animals to arousing and stressful stimuli (psychological, chemical, physical) is known to activate the hypothalamic-pituitary-adrenal axis and sympatho-adrenal-medullary system (Dunn and Kramarcy, 1984; Henry, 1992). Stimulation of the hypothalamic-pituitary-adrenal axis results in enhanced secretion of CRH, adrenocorticotrophic hormone and corticosterone, stimulation of the sympatho-adrenal-medullary system in enhanced plasma noradrenaline and adrenaline levels (Axelrod and Reisine, 1984). These stress hormones cooperate in regulating physiological processes necessary to cope with challenging or threatening situations. Adrenaline, for example, mediates plasma glucose levels during stress (Smythe et al., 1989; Steffens et al., 1984).

As the hypothermia found in the stress-induced hyperthermia procedure is thought to be stress- or anxiety-induced, we studied (Groenink et al., 1994, 1995) whether the hypothalamic-pituitary-adrenal axis and the sympatho-adrenal-medullary system were activated by the stress-induced hyperthermia procedure in group-housed mice.

The temperature curve displayed the expected hyperthermia, leading to about 1.5 °C increase 10 min after the start of the experiment. A normal return to baseline in approximately 1 h occurred as observed previously (Fig. 1A). Plasma levels of adrenocorticotrophic hormone, corticosterone and glucose were enhanced at 10 and 30 min and returned to baseline at 60 min. The findings confirm the hypothesis that the hyperthermia found in mice undergoing the stress-induced hyperthermia test is a stress-mediated response.

The results on body temperature show the normal temperature increase (approximately 1.5 °C) after 10 min, as described previously (Borsini et al., 1989; Zethof et al., 1994). Similarly, the enhanced temperature returns to baseline in a time-dependent manner, as found before (Zethof et al., 1994), thereby confirming that this stress-induced hyperthermia experiment completely fits the range of previous experiments (Zethof et al., 1991, 1994).

The stress-induced hyperthermia is accompanied by increases in plasma concentrations of adrenocorticotrophic hormone, corticosterone and glucose. Both plasma adrenocorticotrophic hormone and corticosterone concentrations were markedly enhanced 10 min after the first temperature measurement. This coincides with the maximum temperature increase. Maximal hyperthermia is always found after 8–10 min (Zethof et al., 1994), which may indicate that stress-induced hyperthermia develops rather slowly. The question remains whether stress intensity also gradually increases from 0 to 10 min. Measuring plasma adrenocorticotrophic hormone, as a fast stress indicator, between time point 0 and 10 may answer this question. Plasma glucose levels were enhanced after 10 and 30 min and were returning to baseline at 60 min. Hyperglycemia is thought to be mainly induced by circulating adrenaline released from the adrenal medulla (Smythe et al., 1989; Steffens et al., 1984) and thus may well reflect sympatho-adrenal-medullary system activity, which is also indicated by the lack of correlation between plasma corticosterone and glucose levels. Therefore, we concluded that the increase in temperature,

induced by the stress-induced hyperthermia test procedure, is clearly associated with activation of the hypothalamic-pituitary-adrenal axis and the sympatho-adrenal-medullary system. Corticotropin-releasing hormone, the first released factor after stimulation of the hypothalamic-pituitary-adrenal axis, has been reported to possess thermogenic effects, which were dependent on sympathetic activation of brown adipose tissue (Rothwell, 1990).

It has been reported that the increase in plasma corticosterone, catecholamine and glucose concentrations, induced by stress or anxiety, can be antagonized by anxiolytics, e.g. benzodiazepines (De Boer et al., 1991; Eisenberg, 1993). SIH in mice can also be antagonized by these anxiolytics, e.g. benzodiazepine receptor agonists (Borsini et al., 1989; Lecci et al., 1990a,b; Zethof et al., 1991, 1994) and 5-HT_{1A} receptor agonists (Lecci et al., 1990a,b; Zethof et al., 1991, 1994). We investigated whether the increase in plasma adrenocorticotrophic hormone and corticosterone levels, induced by the stress-induced hyperthermia procedure in mice, could be antagonized by anxiolytics that also antagonize stress-induced hyperthermia, viz. the benzodiazepine receptor agonist, diazepam (3–12 mg/kg p.o.) and the selective 5-HT_{1A} receptor agonist, flesinoxan (Groenink et al., 1996).

Vehicle-injected mice showed increases in body temperature, plasma adrenocorticotrophic hormone and corticosterone levels following the stress-induced hyperthermia procedure, as described for noninjected mice (Groenink et al., 1994). The increase in plasma glucose levels, induced by the stress-induced hyperthermia procedure, as found in a previous study (Groenink et al., 1994), could not be replicated in the present study. However, the stressor-decapitation interval (10 min) may have influenced the results, as a previous study showed that maximal glucose responses were reached 30 min after starting the stress-induced hyperthermia procedure (Groenink et al., 1994). Under basal nonstress conditions, diazepam reduced the body temperature but had no significant effects on plasma adrenocorticotrophic hormone, corticosterone and glucose levels. Although the plasma corticosterone levels of the 12 mg/kg diazepam-treated group were markedly enhanced, this tendency was not reflected in the plasma levels, but this may be due to the injection-test interval. Plasma adrenocorticotrophic hormone levels were probably returned to baseline values 1 h after injection. Nevertheless, our results indicate that basal effects of diazepam on the activity of the hypothalamic-pituitary-adrenal axis and on plasma glucose levels in mice were not as robust as in rats (Najim et al., 1987; Pericic et al., 1984).

Diazepam reduced stress-induced hyperthermia, as reported previously (Borsini et al., 1989; Zethof et al., 1991, 1995). Diazepam did not reduce the stress-induced elevations in plasma adrenocorticotrophic hormone concentration. Moreover, diazepam did not seem to suppress the stress-induced corticosterone secretion either. The inability of diazepam to suppress the stress-induced hypothalamic-pituitary-adrenal axis activation is remarkable, as in rats, benzodiazepines do suppress stress-induced increases in plasma adrenocorticotrophic hormone and corticosterone concentrations (for review, see De Boer et al., 1992). This lack of effect cannot be attributed to the dose range used, as the higher doses of diazepam (6 and 12 mg/kg p.o.) effectively reduced stress-induced hyperthermia. Our results may, therefore, indicate a difference in benzodiazepine receptor involvement in stress regulation between mice and rats.

Flesinoxan enhanced plasma adrenocorticotrophic hormone and corticosterone levels under nonstress conditions. Similar effects in mice have been described for 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT), another 5-HT_{1A} receptor agonist (Matsuda et al., 1990; Nakuno et al., 1992). These results were in agreement with the effect of 5-HT_{1A} receptor agonists in rats (Bagdy et al., 1989; Groenink et al., 1995). No clear effects of flesinoxan on basal plasma glucose levels were found, whereas flesinoxan has been reported to enhance plasma glucose levels in rats (Groenink et al., 1995).

Flesinoxan blocked stress-induced hyperthermia, as described previously (Zethof et al., 1991, 1995). This anxiolytic effect was not paralleled by a reduction of the stress-induced rises in plasma adrenocorticotrophic hormone levels. The plasma corticosterone concentrations of flesinoxan-treated stressed mice did not differ from those of nonstressed mice. This might indicate that flesinoxan blocks the stress-induced hypothalamic-pituitary-adrenal axis actively. However, it is more likely that the corticosterone-enhancing effects of flesinoxan mask the stress-induced corticosterone enhancement.

Although flesinoxan has no clear suppressant effects on stress-induced rises in plasma adrenocorticotrophic hormone and corticosterone, the effects of flesinoxan and stress on the hypothalamic-pituitary-adrenal axis activity were certainly not additive. In rats, additive effects of stress and intrinsic effects of 5-HT_{1A} receptor agonists on hypothalamic-pituitary-adrenal axis activity have been reported (De Boer et al., 1991; Groenink et al., 1995; Korte et al., 1992; Matheson et al., 1988), although suppressant effects at lower doses have also been suggested (Urban et al., 1986). This may suggest a difference in hypothalamic-pituitary-adrenal axis regulation between rats and mice. Parachlorophenylalanine studies in rats support the idea of postsynaptic 5-HT_{1A} receptor involvement in adrenocorticotrophic hormone and corticosterone secretion (Gilbert et al., 1988). In mice, the involvement of presynaptic 5-HT_{1A} receptors in corticosterone secretion has been suggested (Matsuda et al., 1990). A difference in 5-HT regulation of hypothalamic-pituitary-adrenal axis activation would not be that surprising, for example, thermoregulation in rats and mice seems also differently regulated. In mice, hypothermic effects of 5-HT_{1A} receptor agonists are mediated presynaptically, whereas in rats they seem to be mediated postsynaptically (Bill et al., 1991). Further research is needed to examine

whether the 5-HT involvement in hypothalamic-pituitary-adrenal axis activation really differs between rats and mice. Chronic treatment with either diazepam or flesinoxan could be an interesting approach for further research, as both in the diazepam and the flesinoxan experiment, the effects on basal adrenocorticotrophic hormone and corticosterone levels may be confounded with the effects following stress. Chronic drug treatment may reduce these basal effects.

The results of this study also indicate that the hypothalamic-pituitary-adrenal axis is not directly involved in mediating the stress-induced hyperthermia in mice. Corticotropin-releasing hormone, the first released factor after stimulation of the hypothalamic-pituitary-adrenal axis, has been reported to possess thermogenic effects, which were dependent on sympathetic activation of brown adipose tissue (Rothwell, 1990). As the hypothalamic-pituitary-adrenal axis is stimulated during the stress-induced hyperthermia procedure (this study; Rothwell, 1990), it could be possible that the increase in rectal temperature is a direct effect of hypothalamic-pituitary-adrenal axis stimulation. However, this seems not to be the case, as the hypothalamic-pituitary-adrenal axis remains activated, whereas the stress-induced hyperthermia is reduced following diazepam or flesinoxan treatment.

4. Stress-induced hyperthermia in singly housed male and female mice

Since the number of animals used in the stress-induced hyperthermia procedure can be reduced by 90% when switching from the group-housed to the singly housed procedure, practically all recent data on pharmacology, endocrinology and other physiological and neuroanatomical procedures has been gathered in the singly housed procedure. Moreover, the singly housed stress-induced hyperthermia test appears an excellent procedure to measure stress-related parameters in genetically modified mice.

4.1. Males: pharmacology

Basically, a mouse is injected 60 min before the first rectal temperature measurement with a vehicle or a dose of a drug. In this procedure, a second rectal temperature measurement is performed 10 min later, but intervals of 15 min also suffice (Spooren et al., 2002).

$GABA_A$-benzodiazepine receptor agonists without specificity for certain subunits of the $GABA_A$-benzodiazepine receptor complex have a specific anxiolytic-like profile in which, independent from intrinsic effects of the drugs on basal temperature, a dose-dependent decrease in stress-induced hyperthermia (ΔT) is found, for example, illustrated by diazepam (Table 1). This effect could be antagonized by flumazenil (Olivier et al., 2002), which on itself did not influence stress-induced hyperthermia, indicating the specificity of the $GABA_A$-benzodiazepine receptor involvement. This holds also for other benzodiazepine receptor agonists, including chlordiazepoxide and oxazepam, but subunit-specific benzodiazepine-receptor agonists, including zolpidem (Depoortere et al., 1986), alpidem (Langtry and Benfield, 1990; Zivkovic et al.,1990) and bretazenil were not, or only marginally, anxiolytic in this procedure.

Table 1
$GABA_A$-benzodiazepine receptor complex modulating drugs and stress-induced hyperthermia in singly housed male mice

Drug (route)	Dose (mg/kg)	T_1 (°C)	ΔT (°C)
Diazepam (p.o.)	0	37.7	0.78
	3	37.2	0.30[a]
	6	36.8[a]	− 0.03[a]
	12	36.1[a]	− 0.04[a]
Chlordiazepoxide (p.o.)	0	37.1	0.94
	3	37.3	0.67
	10	37.3	0.11[a]
	30	36.6[a]	− 0.46[a]
Alprazolam (p.o.)	0	37.2	1.00
	0.3	37.2	0.74
	1	36.4[a]	0.35[a]
	3	35.9[a]	− 0.06[a]
Oxazepam (p.o.)	0	37.6	0.74
	0.3	37.6	0.77
	1	37.4	0.68
	3	37.3	− 0.21[a]
Zolpidem (p.o.)	0	37.4	0.71
	3	37.2	0.72
	10	37.1	0.59
	30	36.7[a]	0.41[a]
Alpidem (p.o.)	0	37.4	0.71
	3	37.5	0.78
	10	37.6	0.54
	30	36.3[a]	0.26[a]
Bretazenil (p.o.)	0	37.8	0.94
	3	37.6	0.99
	10	37.7	0.94
	30	36.8[a]	1.19
Alcohol (p.o.)	0	37.3	0.66
	2000	37.2	0.56
	4000	36.4[a]	− 0.12[a]
Phenobarbital (p.o.)	0	37.8	0.94
	30	37.2	0.59
	100	36.0[a]	1.56[a]
	300	36.7[a]	− 0.60[a]
Pentylenetetrazol (s.c.)	0	37.4	0.83
	7.5	37.1	0.66
	15	37.3	0.60
	30	35.9[a]	0.88
FG7142 (p.o.)	0	37.3	0.68
	1	37.4	0.46
	10	37.0	0.71
Baclofen (p.o.)	0	37.8	0.94
	1	38.6	0.60
	3	38.3	0.61
	10	35.2[a]	0.21[a]
Flumazenil (p.o.)	0	37.9	0.59
	3	37.8	0.78
	10	37.7	0.89
	30	38.3	0.55

T_1 is measured 60 min after oral or subcutaneous drug administration. ΔT is $T_2 - T_1$ in which T_2 is the rectal temperature measured 10 min after T_1.
[a] Indicates a significant difference from the vehicle treatment ($p < 0.05$).

Table 2
Effects of serotonergic drugs on stress-induced hyperthermia in singly housed male NMRI mice

Drug (route)	Dose (mg/kg)	T_1 (°C)	ΔT (°C)
Buspirone (p.o.)	0	37.1	0.70
	10	37.4	0.62
	20	37.2	0.57
	40	37.2	0.35[a]
	60	37.1	0.29[a]
Ipsapirone (p.o.)	0	37.1	0.78
	10	37.5	0.75
	20	37.7	0.46
	40	37.7	0.39[a]
	60	37.4	0.29[a]
8-OH-DPAT (s.c.)	0	37.3	0.83
	1	37.1	0.49[a]
	3	37.0	0.11[a]
	10	36.6[a]	0.01[a]
Flesinoxan (p.o.)	0	37.3	0.91
	0.1	37.1	0.71
	0.3	37.2	0.67[a]
	1	37.1	0.44[a]
	3	37.0	0.29[a]
	10	36.7[a]	−0.06[a]
WAY-100,639 (s.c.)	0	37.4	0.69
	0.001	37.3	0.87
	0.01	37.5	0.80
	0.1	37.6[a]	0.88
	1	37.7[a]	0.72
	10	36.9	1.15[a]
S-UH301 (s.c.)	0	37.7	0.58
	1	37.9	0.55
	3	37.8	0.46
	10	37.8	0.49
	30	37.6	0.42
DU125530 (p.o.)	0	37.1	0.64
	3	37.0	0.84
	10	37.1	0.86
	30	36.9	0.68
RU24969 (p.o.)	0	37.5	0.67
	3	37.2[a]	0.63
	10	37.0[a]	0.25[a]
	30	37.2	−0.04[a]
TFMPP (p.o.)	0	37.2	0.81
	3	36.4[a]	1.26
	10	36.4[a]	1.10
	30	36.6[a]	0.33[a]
Anpirtoline (p.o.)	0	38.1	0.94
	1	37.5[a]	1.26[a]
	3	37.1[a]	1.35[a]
	10	36.1[a]	0.88
mCPP (p.o.)	0	37.1	0.94
	3	37.0	1.18
	10	37.4	0.77
	30	37.2	0.62
DOI (p.o.)	0	37.4	0.77
	1	37.5	0.70
	3	37.4	0.72
	10	37.0	0.61
MCPB (p.o.)	0	37.5	0.75
	1	37.9[a]	0.52
	3	37.6	0.69
	10	37.6	0.82
D,L-Propranolol (p.o.)	0	37.4	0.63
	10	36.5	0.80
	20	36.9[a]	0.76
	40	36.7[a]	0.71
Ketanserine (p.o.)	0	37.4	0.66
	1	37.2	0.93
	3	36.3[a]	0.71
	10	35.3[a]	0.39
Ritanserine (p.o.)	0	37.4	0.85
	3	37.2	0.76[a]
	10	36.7[a]	0.66[a]
	30	36.4[a]	0.42[a]
Ondansetron (i.p.)	0	37.3	0.63
	0.001	37.5	0.63
	0.01	37.8[a]	0.70
	0.1	37.4	0.75
	1	37.5	0.66
D,L-Fenfluramine (i.p.)	0	37.3	0.63
	3	37.5	0.62
	10	37.3	0.42[a]
	30	36.9	0.36[a]

T_1 is measured 60 min after oral, intraperitoneal or subcutaneous drug administration. ΔT is $T_2 - T_1$ in which T_2 is the rectal temperature measured 10 min after T_1.

[a] Indicates a significant difference from the vehicle treatment ($p < 0.05$).

Alcohol showed an anxiolytic-like effect, although at a fairly high dose, that also induced hypothermia. Phenobarbital had a somewhat abnormal dose–response effect. At an intermediate dose (100 mg/kg), it reduced T_1, accompanied by an enhanced ΔT, but at the highest dose tested, ΔT was decreased. Pentylenetetrazol had no anxiolytic-like activity. Baclofen had some anxiolytic activity but only at the highest dose tested, accompanied by a drastic hypothermia.

Flesinoxan, a potent, full and selective 5-HT_{1A} receptor agonist also displayed a nice dose-dependent anxiolytic-like response (Table 2). 8-OH-DPAT, also a full and potent 5-HT_{1A} receptor agonist, showed a similar profile, although it is extremely less potent in this procedure than in other anxiolytic tests. This is probably due to the short half-life of 8-OH-DPAT when administered via a systemic route. In contrast to the dramatic hypothermic effects of comparable intravenous-doses of 8-OH-DPAT (Zuideveld et al., 2001, 2002), the present set-up did only induce a slight hypothermia at the highest dose tested (10 mg/kg s.c.), which is probably comparable to the 1 mg/kg i.v. dose used in Zuideveld's studies. Partial 5-HT_{1A} receptor agonists (buspirone, ipsapirone) were also anxiolytic, but to a lesser extent than the full agonists, and had no hypothermic effects at ΔT-reducing doses.

5-HT_{1A} receptor antagonists [WAY-100,635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-*N*-(2-pyridinyl) cyclohexanecarboxamide), S-UH301 ((*S*)-5-fluoro-8-hydroxy-2-(di-*n*-propylamino)-tetralin) and DU125530 (2-[4-[4-(7-chloro)-2,3-dihydro-1,4-benxodioxin-5-yl-piperazinyl] butyl]-1,2-benzisothiazol-3(2H)-one-1,1-dioxide)] have no effects on basal body temperature (T_1) or ΔT at relevant doses used in antagonist experiments. WAY-100,635 had some small enhancing effects on T_1 at intermediate doses

that probably, upon replication, would not replicate. At the extreme high dose of 10 mg/kg s.c. of WAY-100,635, ΔT is enhanced, but at such doses, probably other mechanisms are involved besides the 5-HT_{1A} receptor blockade.

RU24969 [5-methoxy-3-(1,2,5,6-tetrahydro-4-pyridinyl) piperazine] and TFMPP, both mixed 5-$HT_{1A/1B}$ receptor agonists, have anxiolytic-like activity and some slight hypothermic effects (T_1). Because anpirtoline, a rather selective 5-HT_{1B} receptor agonist, has no anxiolytic-like effects, it can be concluded that the anxiolytic effects of RU24969 and TFMPP are probably mediated via the 5-HT_{1A} receptor. Anpirtoline, at intermediate dosing (1 and 3 mg/kg), enhanced ΔT, suggestive of anxiogenic effects. However, because the basal temperature T_1 at these doses was slightly decreased, it also could be an artefact.

The 5-HT_2 receptor agonists mCPP and DOI did not affect ΔT or T_1 at any of the test doses. Similarly, meta-chlorophenylbiguanide (MCPB), a 5-HT_3 receptor agonist, did not affect ΔT and T_1. D,L-Propranolol, a β-adrenoceptor antagonist and partial 5-HT_1 receptor agonist, had no anxiolytic-like activity, although it exerted some intrinsic hypothermic effects. Ketanserine, a 5-$HT_{2A/2C}$ receptor antagonist, had no effect on ΔT, but decreased T_1 at higher doses. Ritanserine, also a 5-$HT_{2A/2C}$ receptor antagonist, had intrinsic hypothermic effects (T_1) and simultaneously also had a relatively small anxiolytic-like effect on ΔT. Ondansetron had no effects in stress-induced hyperthermia, whereas D,L-fenfluramine had anxiolytic-like activity, without influencing the basal body temperature.

Table 3 shows the effects of a number of antidepressant in stress-induced hyperthermia. Practically all antidepressants have no anxiolytic-like activity in this procedure. Only fluoxetine at an intermediate dose, mianserine at the highest dose and tianeptine have some ΔT-decreasing activity. In general, all antidepressants do not exert strong and dose-dependent hypothermic effects.

Drugs acting on various dopaminergic mechanisms are shown in Table 4. Chlorpromazine has strong hypothermic effects, up to almost 7 °C at the 30 mg/kg dose. Although there are significant effects on ΔT at these doses, these values are presumably highly unreliable because of the severe disturbance in temperature regulation at this level of core body temperature. A similar pattern is seen after clozapine treatment. In contrast, the neuroleptic and dopamine D_2 receptor antagonist haloperidol shows some selective anxiolytic-like effects at the highest dose tested, which is not accompanied by severe reduction of T_1. Apomorphine, a nonselective dopamine receptor agonist has no intrinsic effects on ΔT, whereas at the higher doses (0.5 and 1 mg/kg), it slightly reduces T_1. The selective dopamine D_1 receptor antagonist SCH23390 decreased both T_1 and ΔT at the highest dose tested, whereas the selective dopamine D_1 receptor agonist SKF38.393 [(±)-2,3,4,5-tetrahydro-1-phenyl-1H-benzazepine-7,8-diol] had no effects on stress-induced hyperthermia. D,L-Amphetamine decreased T_1 by 2.4 °C only at the intermediate dose of 10 mg/kg, which had returned to normal at the highest dose of 30 mg/kg. No effects were present on ΔT.

Table 3
Antidepressant drugs and stress-induced hyperthermia in singly housed male mice

Drug (route)	Dose (mg/kg)	T_1 (°C)	ΔT (°C)
Imipramine (p.o.)	0	37.4	0.97
	10	37.7	0.55
	30	37.9	0.55
Amitriptyline (p.o.)	0	37.2	0.76
	3	37.1	0.95
	10	36.8	0.98
	30	36.4[a]	0.81
Desimipramine (p.o.)	0	37.4	0.88
	3	37.4	0.63
	10	37.2	0.96
	30	37.1	0.55
Clomipramine (p.o.)	0	37.1	0.82
	3	37.0	0.81
	10	37.2	0.72
	30	36.6	0.64
Fluoxetine (p.o.)	0	37.2	0.81
	3	37.4	0.61
	10	37.3	0.45[a]
	30	37.3	0.49
Fluvoxamine (p.o.)	0	37.4	0.87
	3	37.1	0.84
	10	37.2	0.61
	30	37.2	0.81
Mianserine (p.o.)	0	37.3	0.70
	3	37.5	0.64
	10	37.2	0.55
	30	36.5[a]	0.34[a]
Nomifensine (s.c.)	0	37.6	1.01
	0.3	37.8	0.82
	1	37.3	0.94
	3	36.5[a]	0.85
Clorgyline (p.o.)	0	37.3	0.70
	10	36.8[a]	0.74
	30	37.4	0.76
Tianeptine (p.o.)	0	37.3	0.87
	3	37.3	0.87
	10	37.6	0.49[a]
	30	38.0	0.46[a]

T_1 is measured 60 min after oral or subcutaneous drug administration. ΔT is $T_2 - T_1$ in which T_2 is the rectal temperature measured 10 min after T_1.
[a] Indicates a significant difference from the vehicle treatment ($p < 0.05$).

Table 5 illustrates the effects of drugs with various pharmacological mechanisms in stress-induced hyperthermia. Δ^9-Tetrahydrocannabinol (Δ^9-THC) had, up to a dose of 10 mg/kg, no effects, whereas CP55940 [(−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol], a cannabinoid 1 receptor agonist, had a strong hypothermic effect. Morphine had, at the intermediate dose of 3 mg/kg, some anxiolytic-like effects, but at a higher dose, which on itself decreased T_1; this effect was no longer present. Aspirin (acetylsalicylic acid) had, up to 300 mg/kg, no effect on any measure of stress-induced hyperthermia. Clonidine, an α_2-adrenoceptor agonist, strongly reduced T_1 without effects on ΔT. Prazosine, an α_1-adrenoceptor antagonist, reduced both T_1 and ΔT.

Table 4
Effects of dopaminergic drugs and stress-induced hyperthermia in singly housed NMRI mice

Drug (route)	Dose (mg/kg)	T_1 (°C)	ΔT (°C)
Chlorpromazine (p.o.)	0	37.2	0.76
	3	36.8	0.80
	10	33.1[a]	−0.13[a]
	30	30.7[a]	−0.26[a]
Haloperidol (p.o.)	0	37.5	0.76
	0.3	37.2	0.83
	1	37.2[a]	0.62
	3	37.2[a]	0.16[a]
Apomorphine (s.c.)	0	37.4	0.83
	0.25	37.2	0.54
	0.5	36.5[a]	0.61
	1	36.7[a]	0.65
Clozapine (p.o.)	0	37.4	0.88
	3	37.0	1.05
	10	34.5[a]	0.61
	30	29.7[a]	−0.21[a]
SKF38.393 (p.o.)	0	37.6	0.71
	0.3	37.1	0.75
	1	37.3	0.62
	3	37.4	0.62
	10	37.8	0.49
SCH23390 (p.o.)	0	37.5	0.81
	0.3	37.3	0.76
	1	37.1	0.68
	3	36.7[a]	0.51[a]
D,L-Amphetamine (p.o.)	0	37.1	0.78
	3	36.3	0.76
	10	34.7[a]	0.93
	30	37.5	0.54

T_1 is measured 60 min after oral or subcutaneous drug administration. ΔT is $T_2 - T_1$ in which T_2 is the rectal temperature measured 10 min after T_1.
[a] Indicates a significant difference from the vehicle treatment ($p < 0.05$).

Melatonine had a small hyperthermic effect on T_1 that was not accompanied by clear effects on ΔT. Atenolol, a β-adrenoceptor antagonist that does not pass the blood–brain barrier, had no effects at ΔT and only at the highest dose exerted a small hypothermic effect. Dizocilpine (MK801), an irreversible NMDA antagonist, also had no effect on ΔT and had a small hyperthermic effect at the highest dose tested.

4.2. Mutants

The singly housed stress-induced hyperthermia procedure is optimally suited to obtain fast and important information about core body temperature regulation, response to a stressor and the influence of drugs on these parameters. Thus far, stress-induced hyperthermia has hardly been applied in phenotyping of mutant mice. Watanabe et al. (1999) were the first to measure stress-effects on body temperature in mice, with a targeted mutation of the angiotensin AT_2 receptor gene, and found an enhanced stress-induced hyperthermia after an injection stressor (saline intraperitoneal injection), but not after an interleukin-1β injection, indicating a role for the angiotensin AT_2 receptor in the stress-induced hyperthermia mechanism in the brain.

We have applied the singly housed stress-induced hyperthermia procedure to three mutant mice lines: 5-HT_{1A} (1AKO) and 5-HT_{1B} (1BKO) receptor knockouts and corticotropin-releasing hormone overexpressing (CRH-OE) mice.

5-HT_{1A} receptor knockout (KO) mice, bred on three different genetic backgrounds (Heisler et al., 1998; Parks et al., 1998; Ramboz et al.,1998), were all described with an anxious phenotype, found in several anxiety procedures including elevated plus maze, open field and light–dark box. We postulated that such 1AKO mice might show a differential phenotype in the stress-induced hyperthermia test, for example, an elevated basal body temperature and/or a stronger stress-induced hyperthermia response to the

Table 5
Effects of various miscellaneous drugs and stress-induced hyperthermia in singly housed NMRI mice

Drug (route)	Dose (mg/kg)	T_1 (°C)	ΔT (°C)
Δ^9-THC (i.p.)	0	37.5	0.71
	1	37.5	0.676
	3	37.2	0.70
	10	37.4	0.85
CP55940 (i.p.)	0	37.5	0.71
	0.1	37.1	0.71
	0.3	34.9[a]	0.41
	1	30.2[a]	0.70
Morphine (s.c.)	0	37.6	0.81
	0.3	37.8	0.66
	1	37.9	0.73
	3	37.9	0.32[a]
	10	36.3[a]	0.62
Acetylsalicylic acid (p.o.)	0	37.4	0.63
	60	37.1	0.55
	150	37.5	0.52
	300	37.6	0.47
Clonidine (i.p.)	0	37.3	0.97
	0.03	37.3	1.23
	0.1	34.8[a]	1.05
	0.3	34.0[a]	0.66
	1	31.9[a]	0.71
Prazosine (i.p.)	0	37.3	0.97
	0.125	36.8[a]	1.06
	0.25	36.5[a]	0.69[a]
	0.5	36.3[a]	0.51[a]
	1	36.2[a]	0.44[a]
	2	35.3[a]	0.31[a]
Melatonine (p.o.)	0	37.2	0.79
	60	37.5[a]	0.50[a]
	120	37.4[a]	0.54
	240	37.6[a]	0.66
Atenolol (p.o.)	0	37.4	0.79
	3	37.0	0.86
	10	37.1	1.00
	30	36.8[a]	0.66
Dizocilpine (p.o.)	0	37.3	0.68
	0.03	37.2	0.75
	0.1	37.5	0.81
	0.3	37.6[a]	0.71

T_1 is measured 60 min after oral or subcutaneous drug administration. ΔT is $T_2 - T_1$ in which T_2 is the rectal temperature measured 10 min after T_1.
[a] Indicates a significant difference from the vehicle treatment ($p < 0.05$).

stressor. However, 1AKO mice, at least at the 129Sv-genetic background, had no abnormalities in either of these parameters (Patty et al., 2001). Flesinoxan (0.3–3 mg/kg) was able to antagonize stress-induced hyperthermia in wild-type (WT) mice, but not, as expected in the null mutation, in the 1AKO mice. Diazepam (1–4 mg/kg) antagonized stress-induced hyperthermia in both genotypes and no difference between the effects of diazepam in either genotype was found. Because also circadian rhythmicity in body temperature in 1AKO mice was normal (Olivier et al., 2001; Pattij et al., 2002a,b), it appears that the anxious phenotype of this mutant is only present in certain aspects of anxiety.

5-HT_{1B} receptor knockout mice are impulsive, hyperreactive and more aggressive compared to corresponding WT mice (Bouwknecht et al., 2001a,b,c). The singly housed stress-induced hyperthermia procedure has been extensively used in this mutant in order to measure its response to a mild stressor. The stress-induced hyperthermia procedure can also easily be coupled to telemetric recording and generates extremely interesting data. In a number of such studies, it was found that basal core body temperature of 1BKO mice was higher than the corresponding WT (Bouwknecht et al., 2001b), but this appeared as a function of the time of the day. Measurement of core body temperature over the day showed that basal body temperature is lower in KO mice during the morning hours, but higher during the rest of the day (Bouwknecht et al., 2001a). However, stress-induced hyperthermia from KO mice is not different from WT mice (Bouwknecht et al., 2001b), indicating that the response to a mild stressor in these hyperreactive mice is normal. Similarly, also the rise in corticosterone levels associated with the stress-induced hyperthermia stressor is similar in both genotypes (Bouwknecht et al., 2002). The induction of corticosterone release after the 5-HT_{1A} receptor agonist flesinoxan had a similar dose–response curve in both genotypes (Bouwknecht et al., 2001b), confirming the notion that mechanisms underlying the stress-induced hyperthermia are not disturbed in 5-HT_{1B} receptor knockout mice. Extensive pharmacology in the 1BKO mice (benzodiazepine receptor agonists, 5-HT_{1A} receptor agonists, alcohol, dopaminergic receptor ligands) showed no differences between both genotypes (Bouwknecht et al., 2000) and also no differences compared to earlier findings in NMRI mice.

CRH-OE mice show a chronic-stress-like or depressive phenotype: enhanced basal levels of corticosterone, blunted dexamethasone suppression and reduced locomotor activity (Dirks et al., 2000, 2001a,b, 2002; Groenink et al., 2002). In 24-h circadian rhythms measurements, CRH-OE mice showed higher body temperature than WT, particularly in the second half of the light period. However, in stress-induced hyperthermia experiments, neither basal body temperature nor the response to the stressor (ΔT) was different

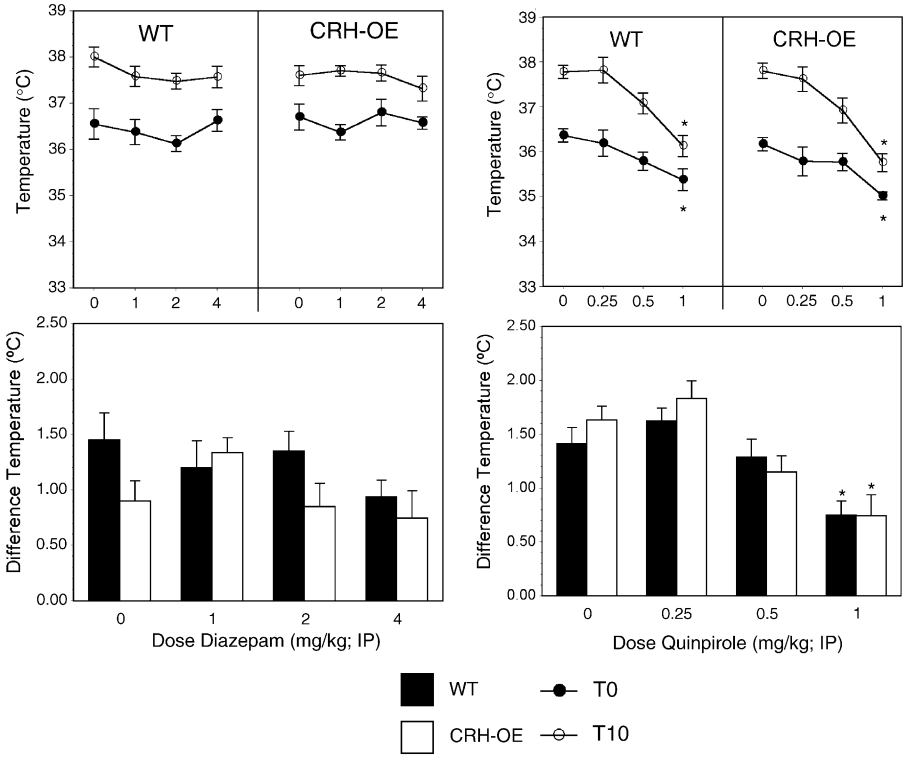

Fig. 2. Male wild-type (WT) and corticotropin-releasing hormone overexpressing (CRH-OE) mice (on a C57BL/6J background) were subjected to a stress-induced hyperthermia procedure. They were injected with either diazepam (left panels; 0, 1, 2 and 4 mg/kg, i.p.) or quinpirole (right panels; 0, 0.25, 0.5 and 1.0 mg/kg, i.p.) 60 min before the rectal procedure. Their rectal temperature was measured twice with an interval of 10 min (T_0 and T_{10}, respectively). T_0 and T_{10} are displayed in the top panels of the figure, the difference ΔT is the stress-induced hyperthermia and is displayed in the lower panels. Significant differences ($p < 0.05$) from vehicle treatment are indicated by *.

between CRH-OE and WT mice. Pharmacological experiments on stress-induced hyperthermia in WT and CRH-OE mice indicated also no differences in the effects of drugs (diazepam, quinpirole; Fig. 2) on either T_1 or ΔT. It can be seen from the results that C57BL/6J mice respond quite insensitive to the drug effects. Therefore, we compared the pharmacological response of three strains, C57BL/6J, 129SvEv and Swiss-Webster, to diazepam (Fig. 3). Diazepam had a strong effect in the 129SvEv strain, decreasing both T_1 and ΔT (from 4 mg/kg onwards), indicating a nice dose-dependent anxiolytic-like effect. In the Swiss-Webster strain, diazepam had no intrinsic effect on T_1, although it still exerted anxiolytic-like effects, but now, only at the highest dose tested (8 mg/kg). In contrast, diazepam up to 16 mg/kg did not influence any measure in C57BL/6J mice. These data clearly indicate the importance of the genetic background in the pharmacological response.

4.3. Females: pharmacology

Thus far, stress-induced hyperthermia has only been tested in male mice, but there is no a priori reason to assume that females have no similar stress response upon a rectal stress procedure. Therefore, we studied female mice of the 129Sv strain in an identical procedure to the male singly housed one and found reliable stress-induced enhancement in temperature of around 0.5–0.7 °C, comparable but slightly lower than males of this strain. Females also could be tested using weekly intervals and their stress-induced hyperthermia remained stable over time (months). Moreover, we also tested female 5-HT_{1B} receptor knockout mice that were made in the same genetic background (129Sv). These KO females showed a comparable profile of responding to the rectal stressor and their basal body temperatures did not differ from the wild type. We tested two compounds in a dose range on these female mice (WT and KO), viz. flesinoxan, a 5-HT_{1A} receptor agonist, and anpirtoline, a 5-HT_{1B} receptor agonist with some 5-HT_{1A} receptor agonistic properties. Flesinoxan, in a dose range of 0.1–3 mg/kg i.p. in both genotypes, dose-dependently decreased both basal body temperature (T_1) and ΔT, showing anxiolytic-like effects of flesinoxan comparable to that obtained in males (Olivier et al., 1998). WAY-100,635 (1 mg/kg) antagonized the flesinoxan-induced decreases in T_1 and ΔT. Anpirtoline also

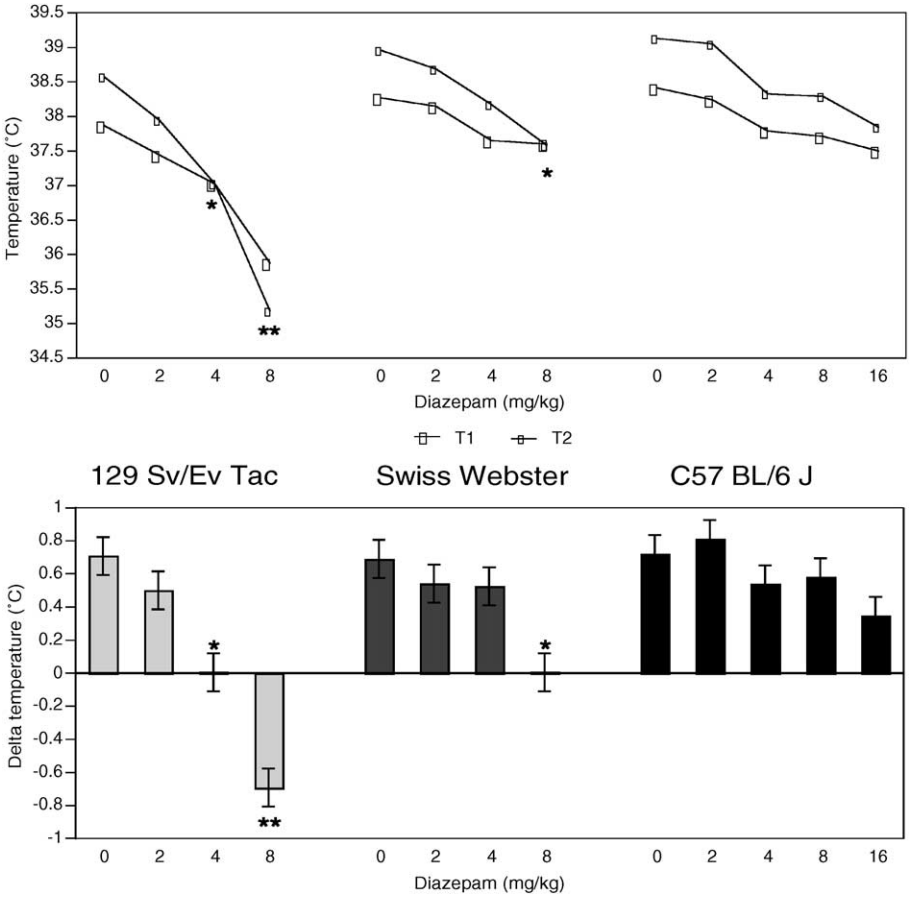

Fig. 3. The effects of diazepam are shown in the stress-induced hyperthermia procedure in three strains of mice, 129SvEv ($N=12$), Swiss-Webster ($N=10$) and C57BL/6J ($N=11$). Animals were injected with all doses of the drug + vehicle once a week, followed 60 min later by two rectal temperature measurements, T_1 and T_2, spaced 10 min. The top panel shows the effects of various doses of diazepam (intraperitoneally injected) on T_1 (core body temperature) and T_2, the enhanced body temperature due to the stress of the first rectal temperature measurement. Stress-induced hyperthermia, the difference between T_2 and T_1 (ΔT) is portrayed in the bottom panel. Significant differences ($p<0.05$) from vehicle treatment are indicated by *.

dose-dependently decreased T_1 in both genotypes, but ΔT showed a U-shaped dose–response curve in both genotypes. At the highest dose tested, ΔT was not different from vehicle treatment in both genotypes. Although slight differences were present between the genotypes, overall, anpirtoline did not differ in its effects on WT and KO mice, suggesting that, in particular, the 5-HT_{1A} agonistic component in its mechanism of action is responsible for the anxiolytic-like activity at the lower dose range. The highest (10 mg/kg) dose was somewhat puzzling, but could be a too high dose to reliably determine stress-induced hyperthermia, because at that dose, the basal temperature was lowered by approximately 4 °C, which also indicates the boundaries of the model in which, at strongly lowered basal temperatures, stress-induced hyperthermia cannot reliably be determined.

5. Discussion

Stress-induced hyperthermia appears a consistent physiological phenomenon when an organism is confronted with a stressor, either physical or psychological. Although the evoking stimuli differ (physical versus psychological), the response of the animal regarding autonomic parameters (body temperature and heart rate, for example) seems to be similar. In the group-housed version of the stress-induced hyperthermia procedure, body temperature rises in anticipation of being picked up and/or the disturbance caused by the experimenter, whereas in the singly housed stress-induced hyperthermia, the temperature rises due to the physical process (handling, rectal measurement) the animal is undergoing. In both procedures, anxiety and fear processes are assumed to play a role in the process of increasing the body temperature following the stressor. There is quite some discussion in literature about the mechanisms and mediators of this stress-induced rise in core body temperature, called psychological fever, or even psychogenic fever by some authors (see discussion in Oka et al., 2001). There is also a discussion whether the rise in body temperature, upon encountering such a stressor, is a real fever or a hyperthermia. If one assumes that fever is reflecting a centrally regulated rise in a set point for core body temperature that can be enhanced for an extended period of time (hours to days), whereas a hyperthermia is an increase in core body temperature without an enhanced set point, one would postulate that stress-induced hyperthermia, as measured in our procedures, is merely a hyperthermia. Oka et al. (2001) give an extensive and elegant overview of arguments whether the psychological stress-induced rise in core temperature (PSRCT) could be a fever or a hyperthermia and conclude that, in particular, PSRCT caused by anticipatory anxiety stress is different from more conventional stress models, like open field stress, because it is not sensitive to cyclooxygenase inhibitors, blocking prostaglandin synthesis. Instead, benzodiazepine receptor agonists and 5-HT_{1A} receptor agonist inhibit the former, but less so the latter models. This is what we also found in our stress-induced hyperthermia model, both in the group-housed and singly housed versions. Acetylsalicylic acid had no influence on ΔT, whereas benzodiazepine receptor agonists, alcohol and 5-HT_{1A} receptor agonists decrease it, suggestive of anxiolytic-like efficacy. In general, the pharmacology found in the group-housed version of stress-induced hyperthermia was largely similar to that found in the singly housed version. There are minor differences, but the experimental situations of the animals (isolated versus group-housed) are different and thermoregulation might demand differential control under these conditions (Gordon et al., 1998). A striking example of this is the effect of amphetamine. In high doses in group-housed conditions, amphetamine induces hyperthermia, eventually leading to death, whereas under isolated conditions, the same doses do have hypothermic or small hyperthermic effects.

We conclude that the singly housed stress-induced hyperthermia procedure is convenient to measure potential anxiolytic-like effects of drugs. At the same time, the procedure measures the intrinsic effects of a drug on core body temperature, an important parameter in the physiological and pharmacological repertoire of psychoactive drugs. The procedure is not able to measure anxiogenic-like effects of drugs. Theoretically, body temperature could be enhanced considerably above the maximal 1.5 °C observed in the stress-induced hyperthermia procedures. Both the stress applied in the stress-induced hyperthermia procedure and temperature increments, induced by drugs or their interactions, are not able to cross this ceiling-like point. This could be a supportive argument for considering stress-induced hyperthermia a hyperthermia and not a fever. In the latter case, one might expect a higher rise in core body temperature.

The stress-induced hyperthermia procedure is easy and can be repeated several times using the same animal and the results are very consistent over time. The procedure can be easily incorporated in screening batteries aiming for testing drugs or mutants, for example, done in a so-called Psychoscreen-battery performed by PsychoGenics. Measurement of the body temperature is not dependent on the motoric activity of the animal and thus has this procedure considerable validity over other anxiety procedures that depend on locomotor performance of the experimental animal, e.g. in the elevated plus maze, the light–dark box or an open field. Moreover, the parameter measured, temperature, is a physiological measure that potentially may reflect other (autonomic) aspects involved in anxiety and fear processes. As such, it is an extremely valuable addition to the armentarium of behavioral pharmacology in the field of anxiety and fear.

References

Axelrod, J., Reisine, T.D., 1984. Stress hormones: their interaction and regulation. Science 224, 452–459.

Bagdy, G., Calogro, A.E., Murphy, D.L., Szemeredi, K., 1989. Serotonin

agonists cause parallel activation of the sympathoadrenomedullary system and the hypothalamo-pituitary-adrenocortical axis in conscious rats. Endocrinology 125, 2664–2669.

Bermant, R.I., Levinson, D.M., Justesen, D.R., 1979. Classical conditioning of microwave-induced hyperthermia in rats. Radio Sci. 14, 201–207.

Bill, D.J., Knight, M., Forster, E.A., Fletcher, A., 1991. Direct evidence for an important species difference in the mechanism of 8-OH-DPAT induced hypothermia. Br. J. Pharmacol. 10, 1857–1864.

Borsini, F., Lecci, A., Volterra, G., Meli, A., 1989. A model to measure anticipatory anxiety in mice? Psychopharmacology 98, 207–211.

Bouwknecht, J.A., Paylor, R., 2002. Behavioral and physiological mouse assays for anxiety: a survey in nine mouse strains. Behav. Brain Res. 136, 489–501.

Bouwknecht, J.A., Hijzen, T.H., van der Gugten, J., Maes, R.A.A., Olivier, B., 2000. Stress-induced hyperthermia in mice: effects of flesinoxan on heart rate and body temperature. Eur. J. Pharmacol. 400, 59–66.

Bouwknecht, J.A., Hijzen, T.H., van der Gugten, J., Maes, R.A.A., Hen, R., Olivier, B., 2001a. Absence of 5-HT_{1B} receptors is associated with impaired motor impulse control in male 5-HT_{1B} knockout mice. Biol. Psychiatry 49, 557–568.

Bouwknecht, J.A., Hijzen, T.H., van der Gugten, J., Maes, R.A.A., Hen, R., Olivier, B., 2001b. Normal corticosterone responses in 5-HT_{1B} receptor knockout mice to stress or 5-HT_{1A} receptor activation. Psychopharmacology 153, 484–490.

Bouwknecht, J.A., van der Gugten, J., Hijzen, T.H., Maes, R.A.A., Hen, R., Olivier, B., 2001c. Corticosterone responses in 5-HT_{1B} receptor knockout mice to stress or 5-HT_{1A} receptor activation are normal. Psychopharmacology 153, 484–490.

Bouwknecht, J.A., Hijzen, T.H., van der Gugten, J., Maes, R.A.A., Hen, R., Olivier, B., 2002. 5-HT_{1B} receptor knockout mice show no adaptive changes in 5-HT_{1A} receptor function as measured telemetrically on body temperature and heart rate responses. Brain Res. Bull. 57, 93–102.

Briese, E., 1995. Emotional hyperthermia and performance in humans. Physiol. Behav. 58, 615–618.

Briese, E., Cabanac, M., 1991. Stress hyperthermia: physiological arguments that it is a fever. Physiol. Behav. 49, 1153–1157.

Briese, E., DeQuijada, M.G., 1970. Colonic temperature of rats during handling. Acta Physiol. Latinoam. 20, 97–102.

Charney, D.S., Woods, S.W., Goodman, W.K., Heninger, G.R., 1987. Serotonin functions in anxiety: II. Effects of the serotonin agonist mCPP in panic disorder patients and healthy subjects. Psychopharmacology 92, 14–24.

Costall, B., Kelly, M.E., Tomkins, D.M., Tyers, M.B., 1989a. Profile of action of diazepam and 5-HT_3 receptor antagonist in the elevated X-maze. Psychopharmacology 3 (10 pp.).

Costall, B., Kelly, M.E., Naylor, R.J., Onaivi, E.S., Myers, M.B., 1989b. Neuroanatomical sites of action of 5-HT_3 agonist and antagonists for alteration of aversive behaviour in the mouse. Br. J. Pharmacol. 96, 325–332.

De Boer, S.F., Slangen, J.L., van der Gugten, J., 1991. Effects of buspirone and chlordiazepoxide on plasma catecholamines and corticosterone levels in stressed and nonstressed rats. Pharmacol. Biochem. Behav. 38, 299–308.

De Boer, S.F., Slangen, J.L., van der Gugten, J., 1992. Brain benzodiazepine receptor control of stress hormones. In: Kvetnansky, R., McCarty, R., Axelrod, J. (Eds.), Stress: Neuroendocrine and Modular Approaches. Gordon & Breach, New York, pp. 719–734.

Depoortere, H., Zivkovic, B., Lloyd, K.G., Sanger, D.J., Perrault, G., Langer, S.Z., Bartholini, G., 1986. Zolpidem, a novel nonbenzodiazepine hypnotic: I. Neuropharmacological and behavioral effects. J. Pharmacol. Exp. Ther. 237, 649–658.

Dirks, A., Groenink, L., lutje Schipholt, M., van der Gugten, J., Hijzen, T.H., Geyer, M.A., Olivier, B., 2000. Reduced startle reactivity and plasticity in transgenic mice overexpressing corticotropin-releasing hormone. Biol. Psychiatry 51, 583–590.

Dirks, A., Pattij, T., Bouwknecht, J.A., Westphal, T.T., van der Gugten, J., Groenink, L., Hijzen, T.H., Oosting, R.S., Hen, R., Geyer, M.A., Olivier, B., 2001a. 5-HT_{1B} receptor knockout, but not 5-HT_{1A} receptor knockout mice, show reduced behavioral reactivity and sensitization by footshocks, as measured with the acoustic startle. Behav. Brain Res. 118, 171–180.

Dirks, A., Groenink, L., Verdouw, P.M., lutje Schipholt, M., van der Gugten, J., Hijzen, T.H., Olivier, B., 2001b. Behavioral analysis of transgenic mice overexpressing corticotropin-releasing hormone in paradigms emulating aspects of stress, anxiety, and depression. Int. J. Comp. Psychol. 14, 123–135.

Dirks, A., Groenink, L., Bouwknecht, J.A., Hijzen, T.H., van der Gugten, J., Ronken, E., Verbeek, J.S., Veening, J.G., Dederen, P.J.W.C., Korosi, A., Schoolderman, L.F., Roubos, E.W., Olivier, B., 2002. Overexpression of corticotropin-releasing hormone in transgenic mice and chronic stress like autonomic and physiological alterations. Eur. J. Neurosci. 16, 1751–1760.

Dunn, R.W., Kramarcy, N.R., 1984. Neurochemical responses in stress: relationship between the hypothalamic-pituitary-adrenal and catecholamine systems. In: Iversen, L.L., Iversen, S.D., Snyder, S.H. (Eds.), Handbook of Psychopharmacology. Drugs, Neurotransmitters and Behavior, vol. 18. Plenum, New York, pp. 455–515.

Dunn, R.W., Corbett, R., Fielding, S., 1989. Effects of 5-HT_{1A} receptor agonists and NMDA receptor antagonists in the social interaction test and the elevated plus maze. Eur. J. Pharmacol. 169, 1–10.

Eisenberg, R.M., 1993. Sound vibration, a non-invasive stress: antagonism by diazepam. Psychopharmacology 110, 467–470.

Gilbert, F., Brazell, C., Tricklebank, M.D., Stahl, S.M., 1988. Activation of the 5-HT_{1A} receptor subtype increases rat plasma ACTH concentration. Eur. J. Pharmacol. 147, 431–439.

Gordon, C.J., Becker, P., Alis, J.S., 1998. Behavioral thermoregulatory responses of single- and group-housed mice. Physiol. Behav. 65, 255–262.

Groenink, L., van der Gugten, J., Zethof, T., Van der Heyden, J., Olivier, B., 1994. Stress-induced hyperthermia in mice: hormonal correlates. Physiol. Behav. 56, 747–749.

Groenink, L., van der Gugten, J., Verdouw, P.M., Maes, R.A.A., Olivier, B., 1995. The anxiolytic effects of flesinoxan, a 5-HT_{1A} receptor agonist, are not related to its neuroendocrine effects. Eur. J. Pharmacol. 280, 185–193.

Groenink, L., van der Gugten, J., Zethof, T.J.J., Van der Heyden, J.A.M., Olivier, B., 1996. Neuroendocrine effects of diazepam and flesinoxan in the stress-induced hyperthermia test in mice. Pharmacol. Biochem. Behav. 54, 249–253.

Groenink, L., Dirks, A., Verdouw, P.M., lutje Schipholt, M., Veening, J.G., van der Gugten, J., Olivier, B., 2002. HPA-axis dysregulation in mice overexpressing corticotropin releasing hormone. Biol. Psychiatry 51, 875–881.

Heisler, L.K., Chu, H., Brennan, T.J., Danao, J.A., Bajwa, P., Parsons, L.H., Tecott, L.H., 1998. Elevated anxiety and antidepressant-like responses in serotonin 5-HT_{1A} receptor mutant mice. Proc. Natl. Acad. Sci. 95, 15049–15054.

Henry, J.P., 1992. Biological basis of the stress response. Integr. Physiol. Behav. Sci. 27, 66–83.

Kenneth, G.A., Whitton, P., Shah, K., Curzon, G., 1989. Anxiogenic-like effects of mCPP and TFMPP in animal models are opposed by 5-HT_{1C} receptor antagonists. Eur. J. Pharmacol. 164, 445–454.

Kleitman, N., 1945. The effect of motion pictures on body temperature. Science 101, 507–508.

Korte, S.M., Bouws, G.A.H., Koolhaas, J.M., Bohus, B., 1992. Neuroendocrine and behavioral responses during conditioned active and passive behavior in the defensive burying/probe avoidance paradigm: effects of ipsapirone. Physiol. Behav. 52, 355–361.

Langtry, H.D., Benfield, P., 1990. Zolpidem: a review of its pharmacodynamic and pharmacokinetic properties and therapeutic potential. Drugs 40, 291–313.

Lecci, A., Borsini, F., Volterra, G., Meli, A., 1990a. Pharmacological val-

idation of a novel animal model of anticipatory anxiety in mice. Psychopharmacology 101, 255–261.
Lecci, A., Borsini, F., Mancinelli, A., D'Aranno, V., Stasi, A., Volterra, G., Meli, A., 1990b. Effect of serotonergic drugs on stress-induced hyperthermia (SIH) in mice. J. Neural Transm. 82, 219–230.
Lecci, A., Borsini, F., Gragnani, L., Volterra, G., Meli, A., 1991. Effect of psychotomimetics and some putative anxiolytics on stress-induced hyperthermia. J. Neural Transm. 83, 67–76.
Marazziti, D., Di Muro, A., Castrogiovanni, P., 1992. Psychological stress and body temperature changes in humans. Physiol. Behav. 52, 393–395.
Matheson, G.K., Gage, D., White, G., Dixon, V., Gipson, D., 1988. A comparison of the effects of buspirone and diazepam on plasma corticosterone levels in the rat. Neuropharmacology 27, 823–830.
Matsuda, T., Kunda, T., Stong, Y.H., Baba, A., Iwata, H., 1990. *p*-Chlorophenylalanine attenuates the pituitary-adrenocortical response to 5-HT_{1A} receptor agonists in mice. Eur. J. Pharmacol. 18, 295–297.
Najim, R.A., Al-Essu, L.Y., Al-Jibouri, L.M., 1987. Benzodiazepine-induced hyperglycemia. Med. Sci. Res. 15, 95–96.
Nakuno, Y., Matsuda, T., Takuma, K., Yoshikawa, T., Baba, A., 1992. Sex differences for tolerance of 5-HT_{1A} receptor-mediated temperature and corticosterone responses in mice. Eur. J. Pharmacol. 219, 339–341.
Oka, T., Oka, K., Hori, T., 2001. Mechanisms and mediators of psychological stress-induced rise in core temperature. Psychosom. Med. 63, 476–486.
Olivier, B., Mos, J., van der Heyden, J.A.M., Tulp, M.Th.M., Slangen, J.L., 1992. Anxiolytic properties of 5-HT3 antagonists: A review. Stress Medicine 8, 117–136.
Olivier, B., Mos, J., van der Heyden, J.A.M., Molewijk, H.E., van Dijken, H.H., Zethof, T., van Hest, A., Tulp, M.Th.M., Slangen, J.L., 1993. Functional correlates of 5-HT receptors, clinical applications and possibilities of serotonergic drugs. In: Claassen, V. (Ed.), Trends in receptor research, vol. 20. Elsevier, Amsterdam, pp. 97–122.
Olivier, B., Zethof, T.J.J., Ronken, E., Van der Heyden, J.A.M., 1998. Anxiolytic effects of flesinoxan in the stress-induced hyperthermia paradigm in singly-housed mice are 5-HT_{1A} receptor mediated. Eur. J. Pharmacol. 342, 177–182.
Olivier, B., van Wijngaarden, I., Soudijn, W., 2000. 5-HT3 receptor antagonists and anxiety: a preclinical and clinical review. Eur. Neuropsychopharmacol. 10, 77–95.
Olivier, B., Pattij, T., Wood, S.J., Oosting, R.S., Sarnyai, Z., Toth, M., 2001. The 5-HT_{1A} receptor knockout mouse and anxiety. Behav. Pharmacol. 12, 439–450.
Olivier, B., Bouwknecht, J.A., Pattij, T., Leahy, C., van Oorschot, R., Zethof, T.J.J., 2002. $GABA_A$-benzodiazepine receptor complex ligands and stress-induced hyperthermia in singly housed mice. Pharmacol. Biochem. Behav. 72, 179–188.
Parks, C.L., Robinson, P.S., Sibille, E., Shenk, T., Toth, M., 1998. Increased anxiety of mice lacking the serotonin 1A receptor. Proc. Natl. Acad. Sci. 95, 10734–10739.
Pattij, T., Groenink, L., Oosting, R.S., van der Gugten, J., Maes, R.A.A., Olivier, B., 2002a. $GABA_A$-benzodiazepine receptor complex sensitivity in 5-HT_{1A} receptor knockout mice on a 129/Sv background. Eur. J. Pharmacol. 447, 67–74.
Pattij, T., Groenink, L., Hijzen, T.H., Oosting, R.S., Maes, R.A.A., van der Gugten, J., Olivier, B., 2002b. Autonomic changes associated with enhanced anxiety in 5-HT_{1A} receptor knockout mice. Neuropsychopharmacology 27, 380–390.
Patty, T., Hijzen, T.H., Oosting, R., van der Gugten, J., Maes, R.A.A., Hen, R., Olivier, B., 2001. Stress-induced hyperthermia in the 5-HT_{1A} receptor knockout mouse is normal. Biol. Psychiatry 49, 569–574.
Peloso, E., Wachulec, M., Satnoff, E., 2002. Stress-induced hyperthermia depends on both time of day and light condition. J. Biol. Rhythms 17, 164–170.
Pericic, D., Lakic, N., Manev, H., 1984. Effect of diazepam on plasma corticosterone. Psychopharmacology 83, 79–81.
Ramboz, S., Oosting, R., Ait Amara, D., Kung, H.F., Blier, P., Mendelsohn, M., Mann, J.J., Brunner, D., Hen, R., 1998. Serotonin receptor 1A knockout: an animal model of anxiety related disorder. Proc. Natl. Acad. Sci. 95, 14476–14481.
Reeves, D.L., Levinson, D.M., Justesen, D.R., Lubin, B., 1985. Endogenous hyperthermia in normal human subjects: experimental study of emotional states (II). Int. J. Psychosom. 32, 18–23.
Renbourn, E.T., 1960. Body temperature and pulse rate in boys and young men prior to sporting contests. A study of emotional hyperthermia: with a review of the literature. J. Psychosom. Res. 4, 149–175.
Rothwell, N.J., 1990. Central effects of CRF on metabolism and energy balance. Neurosci. Biobehav. Rev. 14, 263–271.
Salmi, P., Karlsson, T., Ahlenius, S., 1994. Antagonism by Sch 23390 of clozapine-induced hypothermia in the rat. Eur. J. Pharmacol. 253, 67–73.
Singh, L., Field, M.J., Hughes, J., Menzies, R., Oles, R.J., Vass, C.A., Woodruff, G.N., 1991. The behavioural properties of CI-988, a selective $cholecystokinin_B$ receptor antagonist. Br. J. Pharmacol. 104, 239–245.
Smythe, G.A., Pascoe, W.S., Storlien, L.H., 1989. Hypothalamic noradrenergic and sympathoadrenal control of glycemia after stress. Am. J. Physiol. 256, E231–E235.
Spooren, W.P.J.M., Vassout, A., Neijt, H.C., Kuhn, R., Gasparini, F., Roux, S., Porsolt, R.D., Gentsch, C., 2000. Anxiolytic-like effects of the prototypical metabotropic glutamate receptor 5 antagonist 2-methyl-6-(phenylethynyl)pyridine in rodents. J. Pharmacol. Exp. Ther. 295, 1267–1275.
Spooren, W.P.J.M., Schoeffter, P., Gasparini, F., Kuhn, R., Gentsch, C., 2002. Pharmacological and endocrinological characterisation of stress-induced hyperthermia in singly housed mice using classical and candidate anxiolytics (LY314582, MPEP and NKP608). Eur. J. Pharmacol. 435, 161–170.
Steffens, A.B., Damsma, G., van der Gugten, J., Luiten, P.G.M., 1984. Circulating free fatty acids, insulin, and glucose during chemical stimulation of hypothalamus in rats. Am. J. Physiol. 247, E765–E771.
Urban, J.H., Van de Kar, L.D., Lorens, S.A., Bethea, C.L., 1986. Effect of the anxiolytic buspirone on prolactin and corticosterone secretion in stressed and unstressed rat. Pharmacol. Biochem. Behav. 25, 457–462.
Van der Heyden, J.A.M., Zethof, T.J.J., Olivier, B., 1997. Stress-induced hyperthermia in singly housed mice. Physiol. Behav. 62, 63–70.
Watanabe, T., Hashimoto, M., Okuyama, S., Inagami, T., Nakamura, S., 1999. Effects of targeted disruption of the mouse angiotensin II type 2 receptor gene on stress-induced hyperthermia. J. Physiol. 515, 881–885.
Willner, P., 1991. Behavioural models in psychopharmacology. In: Willner, P. (Ed.), Behavioural Models in Psychopharmacology. Cambridge Univ. Press, Cambridge, pp. 3–18.
Yazumi, T., Ogata, J., Ishida, M., 1989. Analysis of body temperature at different sites in patients having slight fever caused by psychogenic stress. In: Lomax, P., Schonbaum, S. (Eds.), Thermoregulation: Research and Clinical Applications. Karger, Basel, pp. 169–172.
Yokoi, Y., 1966. Effect of ambient temperature upon emotional hyperthermia and hypothermia in rabbits. J. Appl. Physiol. 21, 1795–1798.
Yoshiue, S., Yoshizawa, H., Ito, H., Sekine, H., Nakamura, M., Kanamori, T., Suzuki, H., Yazumi, T., Ogata, J., Ishida, M., 1989. Analysis of body temperature at different sites in patients having slight fever caused by psychogenic stress. In: Lomax, P., Schonbaum, S. (Eds.), Thermoregulation: Research and Clinical Applications. Karger, Basel, pp. 169–172.
Zethof, T.J.J., Van der Heyden, J.A.M., Olivier, B., 1991. A new animal model for anticipatory anxiety? In: Olivier, B., Mos, J., Slangen, J. (Eds.), Animal Models in Psychopharmacology. Birkhäuser-Verlag, Basel, pp. 65–68.
Zethof, T.J.J., Van der Heyden, J.A.M., Tolboom, J.T.B.M., Olivier, B., 1994. Stress-induced hyperthermia in mice: a methodological study. Physiol. Behav. 55, 109–115.
Zethof, T.J.J., Van der Heyden, J.A.M., Tolboom, J.T.B.M., Olivier, B., 1995. Stress-induced hyperthermia as a putative anxiety model. Eur. J. Pharmacol. 294, 125–135.
Zivkovic, N., Morel, E., Joly, D., Perrault, G., Sanger, D.J., Lloyd, K.G.,

1990. Pharmacological and behavioural profile of alpidem as an anxiolytic. Psychopharmacology 23 (Suppl. 3), 108–113.

Zuideveld, K.P., Maas, H.J., Treijtel, N., Hulshof, J., van der Graaf, P.H., Peletier, L.A., Danhof, M., 2001. A set-point model with oscillatory behavior predicts the time-course of 8-OH-DPAT-induced hypothermia. Am. J. Physiol. 281, R2059–R2071.

Zuideveld, K.P., Treytel, N., Maas, H.J., Gubbens-Stibbe, J.M., Peletier, L.A., van der Graaf, P.H., Danhof, M., 2002. A competitive interaction model predicts the effect of WAY-100,635 on the time course of R-8-OH-DPAT-induced hypothermia. J. Pharmacol. Exp. Ther. 300, 330–338.