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Stress-induced hyperthermia as a putative anxiety model

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Abstract

In group-housed mice (ten per cage), mice removed last from their home cage always have higher rectal temperatures than mice removed first from this cage. Stress-induced hyperthermia is calculated as the difference (ΔT) between the basal temperature (mouse number 1) and the end temperature (mouse number 10) when the temperature of the ten mice is sequentially measured using a 1-min interval between rectal measurements. Using this protocol, various drugs, belonging to different pharmacological classes, were tested in order to investigate their putative anxiolytic effect, measured as a decrease in ΔT. Benzodiazepines (diazepam, alprazolam), alcohol, and some (flesinoxan, buspirone), but not all (ipsapirone) 5-HT_{1A} receptor agonists had anxiolytic properties with this protocol. Clonidine (α_2-adrenoceptor agonist) and prazosine (α_1-adrenoceptor antagonist) had, but at high doses, some anxiolytic actions. Antidepressants (desipramine, fluvoxamine, nomifensine, tianeptine, amitriptyline, clomipramine, imipramine), serotonergic ligands (ondansetron, ketanserin, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), fenfluramine, metachlorophenylpiperazine (mCPP), eltoprazine) and various other drugs (phenobarbital, pentetrazol, haloperidol, apomorphine, amphetamine, (+)-*N*-[1-methyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-3(*R*)-yl]-*N'*-(3-methylphenyl)urea (MSD 365260), dizocilpine and acetyl salicylic acid) had no anxiolytic activity. The stress-induced hyperthermia protocol used was unable to detect anxiogenic properties of drugs, probably due to a (physiological) ceiling in the maximal end temperature. The stress-induced hyperthermia protocol with mice can be used to measure anxiolytic properties of drugs and is a fast and robust model which does not need extensive training of animals.

Keywords: Stress; Hyperthermia; Temperature; Anxiety; Benzodiazepine; 5-HT_{1A} receptor agonist; Antidepressant; 5-HT (5-hydroxytryptamine, seroronin); Dopamine

1. Introduction

Behavioural models with animals are necessary for finding new clinically effective drugs and can be helpful for clarifying the neurobiological basis of various psychiatric disorders. The classical animal models for detecting potential anxiolytic activity of drugs were developed decades ago. These models are primarily benzodiazepine-oriented because their validation requires that benzodiazepines should be active in them. In most of these 'classic' animal models of anxiety the effects of some form of aversive stimulus on behaviour is measured. This aversive stimulus can be a footshock or shock applied through the drinking sprout upon water drinking as in the Vogel test (Vogel et al., 1971), footshock coupled to exploration as in the 4-plate test (Aron et al., 1971; Boissier et al., 1968) or various punished responses in conflict procedures as in the Geller-Seifter procedure (Geller and Seifter, 1960). In the light/dark anxiety test (Crawley and Goodwin, 1980) the natural tendency of rodents to avoid brightly lit places is used. Treatment with anxiolytic drugs (benzodiazepines) should ideally facilitate punished (suppressed) behaviour without affecting unpunished behaviour.

Anxiolytic drugs with a non-benzodiazepine mechanism of action, like the 5-HT_{1A} receptor agonist, buspirone, are not or are only very marginally active in these classical models (Barrett, 1991; Barrett and Gleeson, 1991). Buspirone was developed as anxiolytic after

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feedback from clinical trials where it initially was tested as a potential antipsychotic. Since that discovery research into new animal models of anxiety has intensified. These newer animal tests of anxiety preferably have to be sensitive to 5-HT_{1A} receptor agonists and also to the specific serotonin reuptake inhibitors which have also been proven clinically effective as anxiolytics (anti-panic, anti-obsessive compulsive disorder drugs) (Nutt and Glue, 1989).

Recently, a new animal model putatively reflecting anticipatory anxiety was developed: stress-induced hyperthermia in mice (Borsini et al., 1989). The model is based on the finding that when group-housed mice are removed one by one from their home cage, the mice removed last always have higher rectal temperatures than those removed first. This phenomenon is called stress-induced hyperthermia and has been observed, not only in Swiss mice (Borsini et al., 1989) and NMRI mice (Zethof et al., 1994) but also in DBA/2 mice (Rodgers et al., 1994) and has been interpreted as being caused by anticipatory fear of an aversive event (handling). We (Zethof et al., 1994) have extensively evaluated this model with NMRI mice and found stress-induced hyperthermia to be a very robust, reproducible and stable model. The development of stress-induced hyperthermia is time-dependent; it takes approximately 8 min to reach a stable maximal increase of 1.2–1.9°C compared to the basal temperature. Stress-induced hyperthermia is relatively long-lasting, taking approximately 60 min before temperature has returned to its basal level.

The group of Borsini (Borsini et al., 1989; Lecci et al., 1990a,b) has pharmacologically validated this stress-induced hyperthermia model by showing the anxiolytic activity of benzodiazepines and 5-HT_{1A} receptor agonists.

We further validated this model, partly by replicating the earlier findings (Borsini et al., 1989, Lecci et al., 1990a,b) and partly by applying it with other compounds to define its psychopharmacological specificity. We have tested several drugs belonging to different drug classes, e.g. benzodiazepines, alcohol, 5-HT_{1A} receptor agonists, antidepressants and various other psychotropics, with the aim to evaluate stress-induced hyperthermia as a potential animal model of anxiety or stress-related events.

In our procedure, stress-induced hyperthermia (ΔT) is calculated as the difference between the basal T (mouse number 1) and the end T (mouse number 10) of group-housed male mice. Drugs can affect the basal temperature and/or end temperature and several interactions may occur, all having consequences regarding 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 basal T (mouse 1) and on end T (mouse 10) can be envisaged. If a drug does not affect basal T, this drug can affect ΔT in three ways: either no influence (no anxiolytic activity), a decrease in ΔT (interpreted as a real anxiolytic effect), and an increase in ΔT (interpreted as an anxiogenic effect). If a drug has basically a hypothermic effect, a parallel course of basic and end T indicates the absence of any effect, whereas an anxiolytic effect is found if the decline in end T is steeper than the decline in basal T. If the end T is not decreased or is enhanced, this could be interpreted as an anxiogenic effect. When the basal T is enhanced by a drug a similar reasoning can by applied.

Several questions occur when this theoretical model is used. An important one is whether there is a ceiling in the maximal end T that can be reached, i.e. can the end T, which is already 1–2°C above basal T after vehicle treatment, be further enhanced? Extensive evidence suggests that indeed such a ceiling limits the predictability of the model, especially when anxiogenic effects are sought for.

An important parameter in the stress-induced hyperthermia model used is the time interval between injection of the experimental drug and measurement of rectal temperature. In previous work (Zethof et al., 1994) we found that manipulations (injection, handling) of mice in the stress-induced hyperthermia setup lead to a (stress-induced) temperature rise which has declined to baseline after approximately 60 min. Therefore, in the present experiments we have chosen 60 min as the injection–test interval.

2. Material and methods

2.1. General procedures

The procedure used is a modification of that described by Borsini et al. (1989) and was extensively described by Zethof et al. (1994). Mice from one cage were randomly numbered 1–10 (handling) with dye one day prior to the temperature measurement. The rectal temperature of group-housed animals was measured sequentially. After temperature measurement the animals were placed in another cage (i.e. number of mice in test cage decreasing from 10 to 1). Cages were randomly allocated over technicians, times of day (morning–afternoon) and treatments in each experiment. The temperature of all ten mice was measured sequentially by inserting a thermistor probe for a length of 2 cm into the rectum of the mice. Digital recordings of the temperature were made with an accuracy of 0.1°C by means of 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 was measured for 20 s. In all

experiments the time interval between the rectal temperature measurements of individual mice was 1 min.

2.2. Animals

Male NMRI mice (Charles River, Sulzfeld, Germany) weighing approximately 12–14 g upon arrival in the laboratory, were housed in groups of ten per cage (dimensions: 34 × 22 × 15 cm) under non-reversed 12 h light-12 h dark cycle conditions (lights on from 07.00 to 19.00 h). The animals were housed at constant room temperature (21 ± 2°C) and relative humidity (60 ± 10%) with food and water freely available. Experiments were carried out between 9.00 a.m. and 3 p.m. by two trained technicians. The animals could adapt for at least one week to the test room (for detailed experimental setup see Zethof et al., 1994).

2.3. Drug administration and sources

Eight cages per group were used; each cage contained ten male mice. All drugs and vehicle were given orally, subcutaneously or intraperitoneally in a volume of 10 ml/kg body weight. All animals in a cage received the same treatment and were injected within 3 min. After 60 min the temperature measurement were started using a 1-min interval.

The oral vehicle was tragacanth 1% (w/v) and that for intraperitoneal treatment was saline.

Drugs were obtained from the following sources: diazepam · base, apomorphine · HCl, *d,l*-amphetamine · sulphate and pentetrazol · base from O.P.G., Netherlands, alprazolam · base from Upjohn, USA; buspirone · HCl, imipramine · HCl, amitriptyline · HCl, desimipramine · HCl, acetyl salicylic acid and clonidine ·

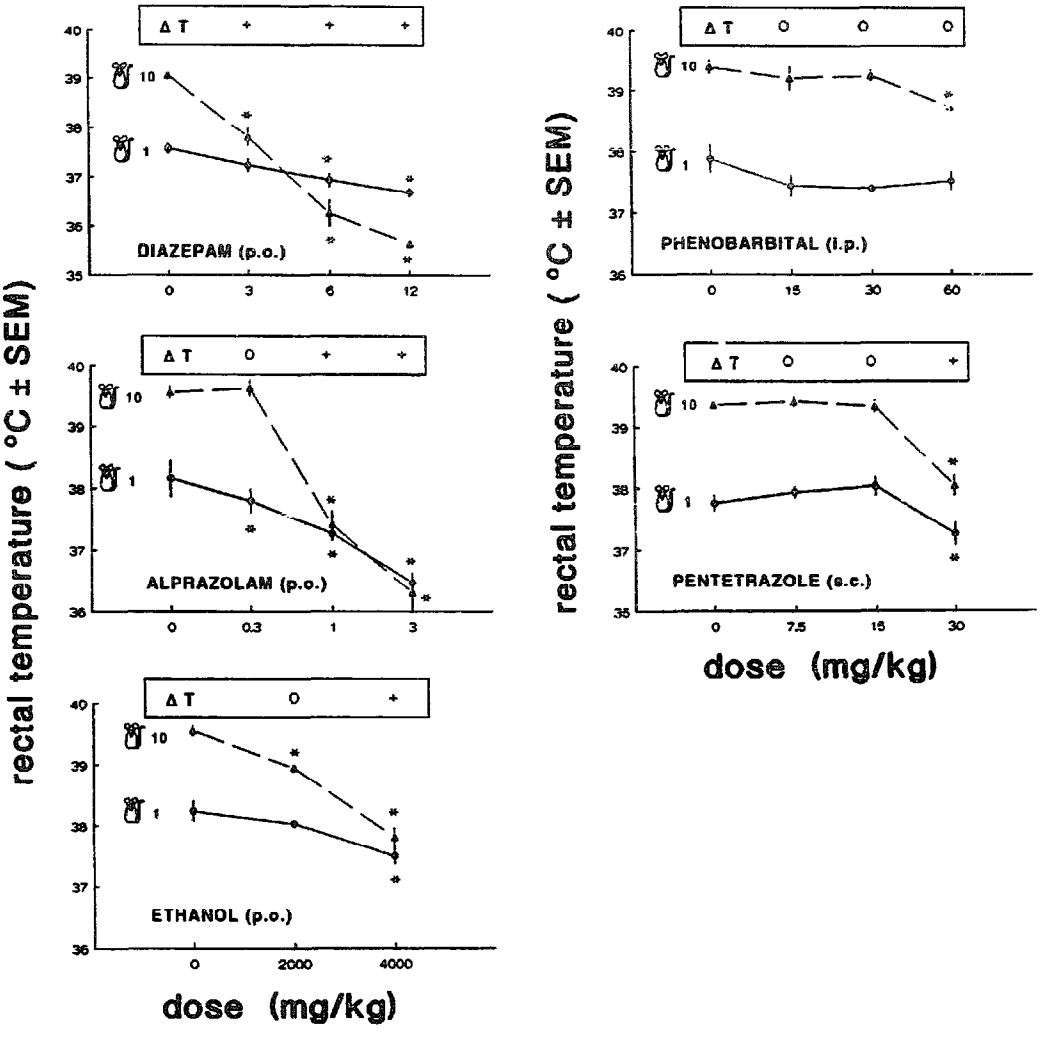

Fig. 1. Effects of GABA-benzodiazepine receptor ligands on stress-induced hyperthermia. The effects of drugs are shown on the temperature of mouse 1 (basal T) and mouse 10 (end T) in group-housed mice. ΔT (at the top) is the difference in temperature between mouse 1 and mouse 10. * ($P < 0.05$) indicates a significant difference from the vehicle (0 mg/kg) dose. +($P < 0.05$) indicates a significant difference in ΔT compared to ΔT after vehicle (0 mg/kg) treatment; 0 indicates no significant effect.

HCl from Sigma, Germany; flesinoxan hydrochloride, ipsapirone · base, ondansetron · base, DOI (1-((2,5)-dimethoxy-4-iodo)-phenyl)-2-amino-propane), eltoprazine · HCl, fluvoxamine · maleate, tianeptine · base and MSD 365260 ((+)-*N*-[1-methyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-3(*R*)-yl]-*N'*-(3-methylphenyl)urea) were synthesized by Solvay Duphar, Netherlands. Ketanserine · tartrate and dizocilpine · maleate (MK 801) were obtained from R.B.I., USA; mCPP (metachlorophenylpiperazine · HCl) from Aldrich Chemicals; *d,l*-fenfluramine · HCl and clomipramine · HCl from BUFA, Netherlands; nomifensine · maleate from Hoechst, Germany; haloperidol · base from Siegfried Zofingen, Switzerland; phenobarbital · base from Brocacef, Netherlands and prazosine · HCl from Pfizer, Belgium.

2.4. Statistics

An individual cage containing ten mice was considered a separate, independent experimental unit. For each individual cage the rise in rectal temperature (ΔT) was calculated as the difference between the fitted value for mouse 10 minus the fitted value for mouse 1 using linear regression analysis of the temperature of all ten mice per cage. The basal temperature (basal T) was defined as the fitted value from mouse 1. The end temperature (end T) was defined as the fitted value from mouse 10. Hereafter, the mean basal T, end T and ΔT for the eight cages were calculated.

After homogeneity of variance for the variables basal T, end T and ΔT was checked between treatments, treatment effects were evaluated by three-way analysis of variance (ANOVA). According to the experimental design the explanatory factors were treatment, time of day (morning or afternoon) and technician (A or B) together with all interactions. If the overall ANOVA appeared significant, t-tests were used to find significant differences between drug doses and vehicle for the three parameters 'basal T', 'end T' and 'ΔT'.

3. Results

3.1. GABA-benzodiazepine ligands (Fig. 1)

The benzodiazepine receptor agonists, diazepam and alprazolam, significantly reduced stress-induced hyperthermia. At 3 mg/kg p.o. diazepam significantly reduced end T while basal T was not affected. At higher doses (6 and 12 mg/kg) both end T and basal T were decreased. ΔT was significantly reduced at all doses tested (3–12 mg/kg). Alprazolam decreased basal T significantly (from 0.3 mg/kg p.o. and higher doses) while end T was significantly decreased at 1 and 3 mg/kg. ΔT was significantly reduced at 1 and 3 mg/kg.

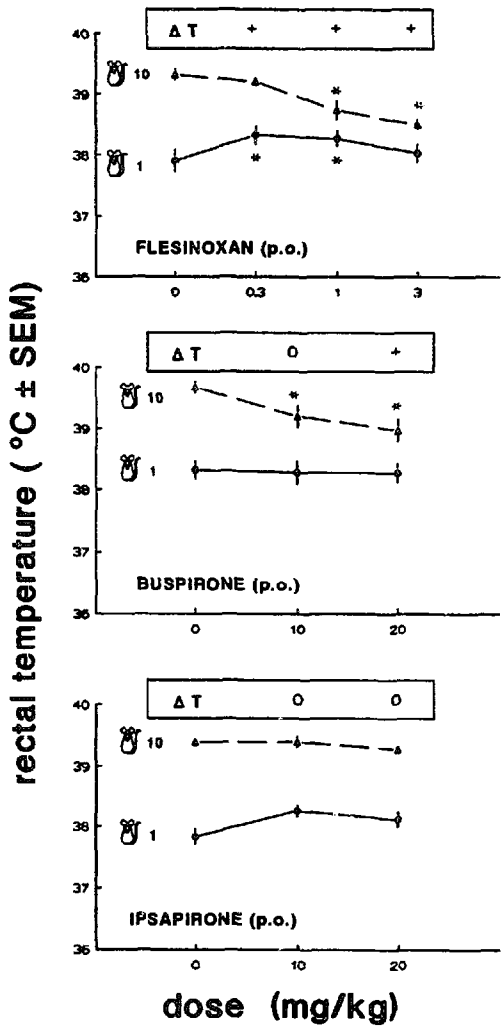

Fig. 2. Effects of 5-HT_{1A} receptor agonists on stress-induced hyperthermia (for further explanation see legend to Fig. 1).

Ethanol dose-dependently reduced end T and basal T. Basal T was significantly reduced at 4 g/kg while end T was significantly reduced at 2 and 4 g/kg. Only at 4 g/kg was a significant decrease of ΔT found. Phenobarbital did not affect basal T, and decreased end T at the highest dose (60 mg/kg) tested, although not resulting in a significantly decreased ΔT at that dose. Pentetrazol had a decreasing effect on basal T and on end T, only at the highest dose (30 mg/kg), although the latter effect was more pronounced than the former, resulting in a decreased ΔT.

3.2. 5-HT_{1A} receptor agonists (Fig. 2)

The 5-HT_{1A} receptor agonist, buspirone, reduced end T at 10 and 20 mg/kg and had no effect on basal T. ΔT was significantly decreased at 20 mg/kg. Ipsapirone did not significantly affect basal T and end T at 10 or 20 mg/kg. No effect was found in ΔT. Flesinoxan dose dependently reduced end T and at the intermediate doses of 0.3 and 1 mg/kg, slightly but

significantly increased basal T. ΔT was significantly decreased at all doses tested (0.3–1 mg/kg).

3.3. Antidepressants (Fig. 3)

Imipramine had no effect on end T whereas basal T was decreased at the highest dose (30 mg/kg) tested. ΔT was significantly enhanced at 3 and 30 mg/kg, but not at 10 mg/kg. Amitriptyline had, only at the highest dose tested, suppressing effects on both basal T and end T, but no effect on ΔT. Desimipramine decreased basal T (only significant at 30 mg/kg) and end T (at 3, 10 and 30 mg/kg), but there was no significant effect on ΔT. Clomipramine had no effect (up to 30 mg/kg) on basal T, end T, or ΔT (data not shown). Fluvoxamine slightly, but significantly enhanced basal T over the whole dose range tested, but had no effect on end T. There was a small but significant decrease in ΔT. Nomifensine (at the highest dose of 3 mg/kg), enhanced basal T and had no effect on end T. At 3 mg/kg nomifensine reduced ΔT. Finally, tianeptine induced a dose-dependent increase in basal T, but had no effect on end T, resulting in a significant decrease in ΔT at 10 and 30 mg/kg.

3.4. Serotonergic ligands (Fig. 4)

Ondansetron, in a dose range of 0.001–0.1 mg/kg i.p., had no effect on any of the temperature parameters. Ketanserine dose dependently decreased basal T and end T, but had no effect on ΔT. DOI enhanced basal T at all three doses, but not dose-dependently, and had no effect on end T, leading to a decrease in ΔT. *d,l*-Fenfluramine significantly enhanced basal T at 3 mg/kg and decreased end T at 30 mg/kg. The decrease in ΔT was significant at all doses but no dose-response relation was observed. Metachlorophenylpiperazine had no significant effects on basal T or end T, but reduced ΔT at the highest dose tested (10 mg/kg). Eltoprazine yielded an unexpected, dose-dependent and U-shaped curve for both basal T and end T, but there was no effect on ΔT.

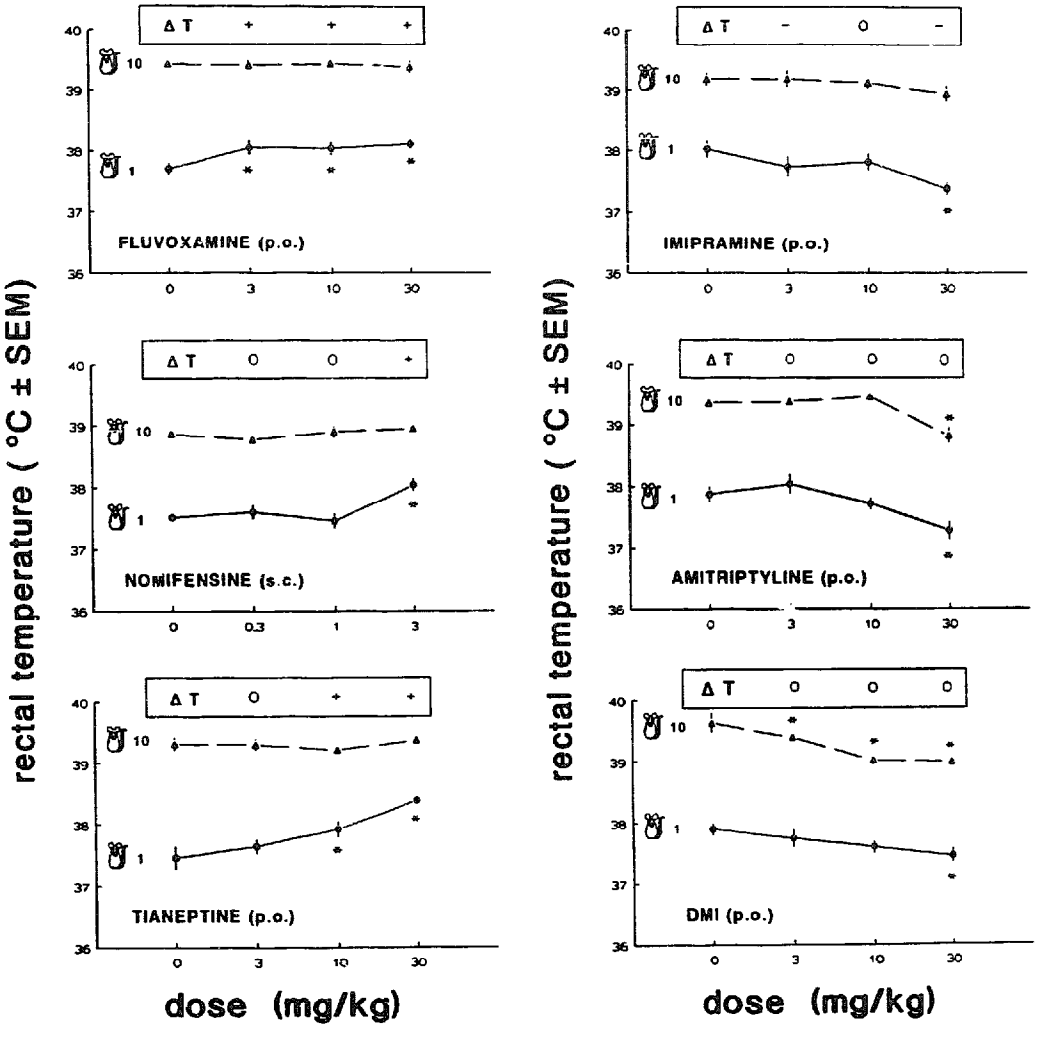

Fig. 3. Effects of various antidepressants on stress-induced hyperthermia (for further explanation see legend to Fig. 1).

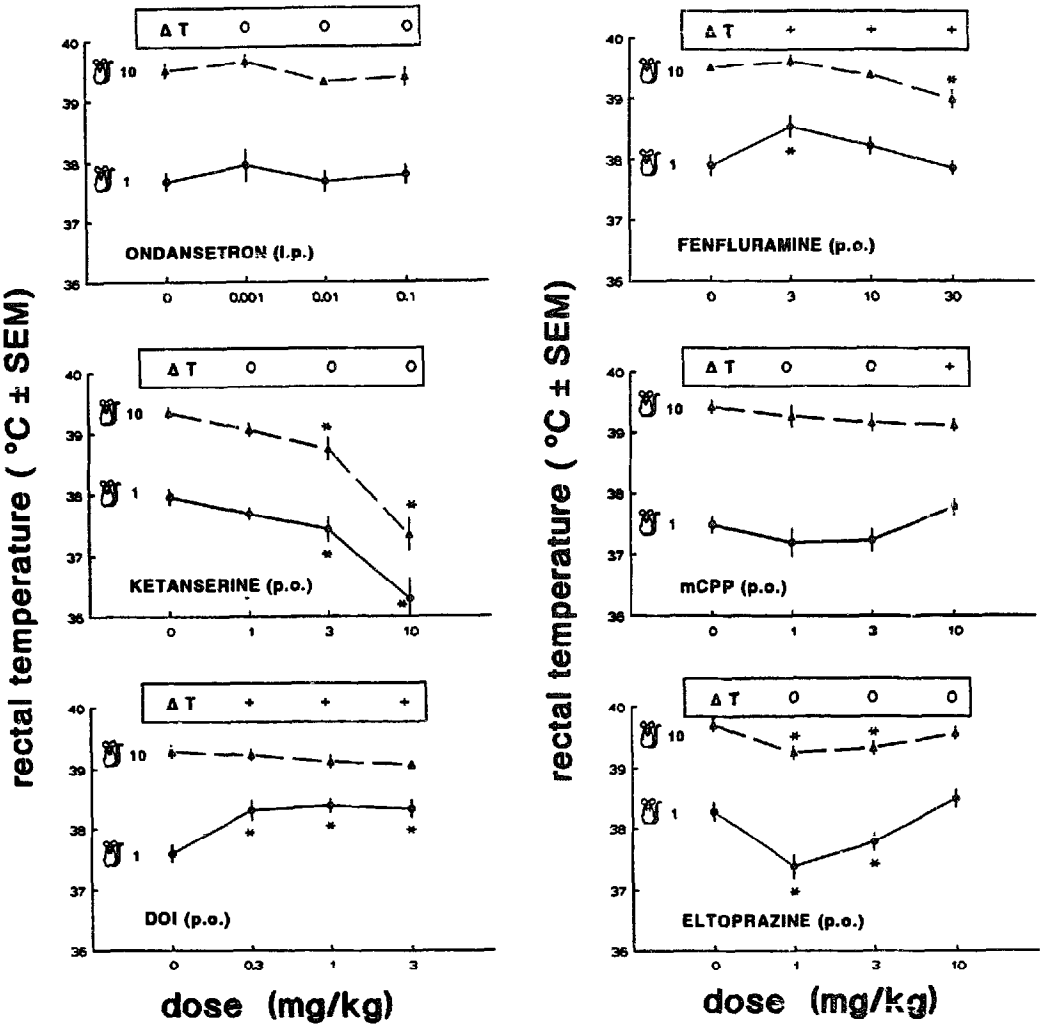

Fig. 4. Effects of various serotonergic ligands on stress-induced hyperthermia (for further explanation see legend to Fig. 1).

3.5. Miscellaneous drugs

Table 1 summarizes the results for members of different classes of psychoactive drugs. Haloperidol had no effect on basal T and decreased end T only at 3 mg/kg. However, no significant effect was found on ΔT. Apomorphine decreased dose dependently both basal T and end T, resulting in an unaffected ΔT. *d,l*-Amphetamine produced a rather peculiar pattern. At doses up to 3 mg/kg (i.p.), *d,l*-amphetamine decreased both basal T and end T, whereas at 10 mg/kg basal T was significantly enhanced, and end T was again normal. Only at 10 mg/kg was ΔT reduced. Clonidine dose dependently reduced basal T and end T. Only at the highest dose tested (1 mg/kg), was there a significant decrease in ΔT. Prazosine dose dependently decreased basal T and end T. At the highest dose (2 mg/kg) ΔT was significantly reduced. MSD 365260 decreased basal T significantly at 100 mg/kg and end T significantly at 10 and 100 mg/kg not resulting in any effect on ΔT. Dizocilpine (MK801) increased basal T at the highest dose tested (0.3 mg/kg) but had no effect on end T and ΔT. Acetyl salicylic acid significantly increased basal T at 300 and 600 mg/kg, significantly decreased end T at 600 mg/kg, resulting in a decreased ΔT at 300 and 600 mg/kg.

4. Discussion

When an animal test is proposed to model an affective process, in this case anxiety, it should ideally have face, construct and predictive validity (Willner, 1991). Stress-induced hyperthermia seems to have high face validity in that 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: Snow and Horita, 1982; Yokoi, 1966) including humans (Marazziti et al., 1992, Yoshiue et al., 1989). A model has construct validity if the mechanism behind the pathology seems to have similar physiological substrates in the animal model

Table 1
Results for various drugs in the stress-induced hyperthermia model

Drug	Dose	Route	Basal T	End T	ΔT
Haloperidol	0	p.o.	37.9 ± 0.1	39.3 ± 0.1	1.4 ± 0.2
	0.3	p.o.	38.0 ± 0.1	39.4 ± 0.1	1.4 ± 0.1
	1	p.o.	38.0 ± 0.1	39.2 ± 0.1	1.1 ± 0.1
	3	p.o.	37.8 ± 0.1	39.0 ± 0.1 [a]	1.2 ± 0.1
Apomorphine	0	s.c.	37.8 ± 0.2	39.4 ± 0.2	1.5 ± 0.2
	0.25	s.c.	37.8 ± 0.1	39.0 ± 0.1 [a]	1.2 ± 0.1
	0.5	s.c.	37.1 ± 0.2 [a]	38.6 ± 0.1 [a]	1.5 ± 0.2
	1	s.c.	36.8 ± 0.3 [a]	37.9 ± 0.2 [a]	1.1 ± 0.3
d,l-Amphetamine	0	i.p.	38.0 ± 0.1	39.3 ± 0.1	1.3 ± 0.1
	1	i.p.	37.9 ± 0.4	38.6 ± 0.3 [a]	0.7 ± 0.3
	3	i.p.	36.9 ± 0.3 [a]	38.2 ± 0.1 [a]	1.3 ± 0.3
	10	i.p.	39.3 ± 0.4 [a]	39.3 ± 0.2	0.0 ± 0.3 [a]
Clonidine	0	i.p.	38.2 ± 0.2	39.6 ± 0.2	1.4 ± 0.1
	0.1	i.p.	37.8 ± 0.1 [a]	39.2 ± 0.1 [a]	1.4 ± 0.1
	0.3	i.p.	37.2 ± 0.1 [a]	38.5 ± 0.1 [a]	1.3 ± 0.2
	1	i.p.	36.3 ± 0.1 [a]	36.8 ± 0.2 [a]	0.5 ± 0.2 [a]
Prazosine	0	i.p.	38.1 ± 0.1	39.1 ± 0.1	1.0 ± 0.1
	0.5	i.p.	37.3 ± 0.1 [a]	37.8 ± 0.1 [a]	0.5 ± 0.2
	1	i.p.	36.8 ± 0.2 [a]	37.6 ± 0.3 [a]	0.8 ± 0.3
	2	i.p.	36.1 ± 0.2 [a]	36.1 ± 0.4 [a]	−0.1 ± 0.4 [a]
MSD 365260	0	i.p.	38.2 ± 0.2	39.5 ± 0.1	1.4 ± 0.2
	1	i.p.	38.2 ± 0.2	39.4 ± 0.1	1.1 ± 0.2
	10	i.p.	37.9 ± 0.2	39.3 ± 0.1 [a]	1.4 ± 0.1
	100	i.p.	37.1 ± 0.2 [a]	38.7 ± 0.2 [a]	1.6 ± 0.3
Dizocilpine	0	p.o.	37.9 ± 0.2	39.4 ± 0.1	1.5 ± 0.2
	0.03	p.o.	37.7 ± 0.1	39.5 ± 0.1	1.9 ± 0.1
	0.1	p.o.	38.1 ± 0.1	39.6 ± 0.1	1.5 ± 0.1
	0.3	p.o.	38.6 ± 0.2 [a]	39.8 ± 0.1	1.2 ± 0.2
Acetyl salicylic acid	0	p.o.	37.9 ± 0.2	39.2 ± 0.1	1.3 ± 0.1
	150	p.o.	38.2 ± 0.1	39.3 ± 0.1	1.0 ± 0.1
	300	p.o.	38.7 ± 0.1 [a]	39.2 ± 0.1	0.5 ± 0.1 [a]
	600	p.o.	38.5 ± 0.1 [a]	38.9 ± 0.1 [a]	0.4 ± 0.1 [a]

Basal T refers to the fitted value for mouse 1, End T refers to fitted value for mouse 10. ΔT refers to difference between fitted value for mouse 1 minus fitted value for mouse 10. [a] $P < 0.05$ (according to t-test after ANOVA) indicates significant difference from vehicle treatment (0 mg/kg).

and in man. Marazziti et al. (1992) found strong indications that stress-related hyperthermia in man uses physiological and endocrinological mechanisms similar to those found in animals. Although temperature regulation is an integrative response to different stimuli at the level of the hypothalamus, it is tempting to hypothesize that similar mechanisms are involved in humans and in animals, including corticotropin-releasing hormone. Corticotropin-releasing hormone is involved in the regulation of a variety of responses occurring in stress and anxiety (Dunn and Berridge, 1990). 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 conform to this requirement.

As already indicated in the Introduction, anxiolytic effects of drugs can be found when the effect on the stress-induced rise in end T (mouse 10) is decreased more than the basal T (mouse 1). In a previous study (Zethof et al., 1994) we have extensively investigated the optimal circumstances for drug testing in this context and have found that an injection test interval of 60 min is necessary to avoid residual effects of the injection procedure on basal T. Using this procedure we were able to observe drugs which decrease, increase or have no intrinsic effects on basal T. Similarly, we were able to detect anxiolytic effects of drugs, i.e. the decreasing effect of a drug is stronger on the temperature of mouse 10 than on that of mouse 1. The predictive validity of a model would improve considerably if it were also sensitive to anxiogenic drugs. The stress-induced hyperthermia model with mice however fails to detect anxiogenic compounds (e.g. pentetrazol, mCPP). We have reason to postulate that under the present circumstances the body temperature of mice cannot be increased further than the 1.0–1.5°C observed. In an extensive range of experiments we found that the end T is more or less fixed, as in all experiments the end T of drug-treated mice was maximally 0.1°C higher than the end T of vehicle-treated mice. This means that an anxiogenic effect, as postulated earlier, cannot be de-

duced from a further enhancement of stress-induced hyperthermia because of a ceiling effect on the maximal reachable temperature. In 29 experiments the average basal T was 37.89 ± 0.04 (range 37.45–38.31) with vehicle, whereas the average end T was 39.38 ± 0.03 (range 38.86–39.70) with vehicle, with an average ΔT of 1.49 ± 0.04 (range 0.97–1.95).

With drug treatment increases in basal T can easily be detected, but increases in end T were few and if present, were non-significant (in the range of 0.1–0.2°C). The only exception, dizocilpine (MK801), induced at all doses a small, but non-significant enhancement compared to vehicle, of the end T, viz. 0.12, 0.20 and 0.36 at 0.03, 0.1 and 0.3 mg/kg p.o. respectively. The ceiling for end T is perhaps best illustrated with *d,l*-amphetamine. The basal T is first dose dependently decreased from 38.02 (0 mg/kg) to 36.92°C (3 mg/kg) followed by a marked increase at 10 mg/kg, to 39.28°C which is significantly higher than the basal vehicle temperature. The end T tends to parallel the basal temperature, except at the 10 mg/kg dose, where the increase seems to be damped by the (physiological?) ceiling. In all the experiments performed by the group of Borsini (Borsini et al., 1989; Lecci et al., 1990a,b,1991) the end temperature increased comparably to those reported here. The only exception was *d*-amphetamine (Lecci et al., 1991). A dose of 10 mg/kg (i.p.) raised the end temperature to 40.1°C but the basal temperature was also strongly increased (39.9°C). Normally, both basal temperature and end temperatures in the present studies and the Lecci experiments are in the same range. Therefore, we think that the effect of amphetamine reflects a toxic phenomenon, leading to a pathologically elevated temperature. There is indeed abundant evidence that amphetamine can lead to lethality in grouped animals (like the experiments described here and in Lecci et al.'s experiments), but not in isolated animals (Chance, 1946, 1947). As we used *d,l*-amphetamine, which is less active than *d*-amphetamine, we could have observed an effect similar to that seen by Lecci et al. (1991) had we used higher doses.

This makes it plausible that the ceiling effect on the end T precludes the measurement of anxiogenic effects. The group of Borsini and colleagues (Borsini et al., 1989; Lecci et al., 1990a) used a somewhat different procedure to measure stress-induced hyperthermia and postulated that they could observe anxiogenic effects. They used groups of 20 mice per cage and found that yohimbine increased the number of hyperthermic mice (8 versus 17 mice). In another study (Lecci et al., 1990b), the possible anxiogenic properties of trifluoromethyl phenylpiperazine (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 (Kennett et al., 1989), failed to show an anxiogenic profile in their 20-mouse design. In our 10-mouse design we also did not observe an increased number of hyperthermic mice after administration of the putative anxiogenics, pentetrazol and mCPP. The detection of anxiogenic effects of drugs is thus difficult, probably due to the more or less fixed maximum reachable temperature. Another implication of the fixed end T will be that ΔT can be decreased due to an increased basal T. In this case, the decreased ΔT is probably not a real anxiolytic effect but a false-positive result due to the increased basal T in combination with the fixed end T. This implies that an increased basal T interferes with stress-induced hyperthermia, resulting in non-specific results.

Therefore, it can be concluded that any anxiolytic activity of drugs can easily be detected 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 the benzodiazepines (diazepam and alprazolam), 5-HT_{1A} receptor agonists (flesinoxan, buspirone but not ipsapirone) and ethanol are active to block stress-induced hyperthermia. In contrast to the benzodiazepines (diazepam, alprazolam) which completely abolish stress-induced hyperthermia, the 5-HT_{1A} receptor agonists only partially (flesinoxan, buspirone) or do not block (ipsapirone) stress-induced hyperthermia. This may point to differential mechanisms (benzodiazepines, 5-HT_{1A} receptors) regulating stress-induced hyperthermia, but may also be due to the intrinsic hypothermic effects of benzodiazepines, which are lacking in 5-HT_{1A} receptor agonists. Moreover, the partial inhibition of stress-induced hyperthermia by the 5-HT_{1A} receptor agonists may reflect the intrinsic activity of these compounds at the 5-HT_{1A} receptor. Flesinoxan is a full 5-HT_{1A} receptor agonist, whereas buspirone and ipsapirone are partial receptor agonists. Although this does not explain the ineffectivity of ipsapirone to lower stress-induced hyperthermia, it may contribute to the lack of effect.

A further remarkable effect of 5-HT_{1A} receptor agonists on stress-induced hyperthermia is their lack of effect on basal temperature. Flesinoxan even shows (at 0.3 and 1 mg/kg p.o.) a small hyperthermia. Although it is generally found (e.g. Bill et al., 1989; Martin et al., 1992) that activation of (presynaptic) 5-HT_{1A} receptors induces a hypothermic effect, most experiments are performed on isolated mice or sequential temperature measurements are performed on the same mouse, thereby themselves inducing stress-induced hyperthermia (cf. Olivier et al., 1994a). Under our experimental conditions, i.e. group-housed mice which are sequentially measured and removed from the housing cage, 5-HT_{1A} receptor agonists do not lead to hypothermia,

unless extra high dosages are given. Apparently, group housing creates a different homeostatic mechanism than does isolation.

In our stress-induced hyperthermia model phenobarbital had no anxiolytic activity up to 60 mg/kg i.p. Phenobarbital, acting through the benzodiazepine/GABA receptor complex (Sieghart, 1992), showed anxiolytic activity at 20 mg/kg i.p. in the stress-induced hyperthermia model of Lecci et al. (1990a). We have no explanation for this discrepancy.

Antidepressants are inactive to block stress-induced hyperthermia. The mixed noradrenergic/serotonergic reuptake inhibitors imipramine, amitriptyline, desimipramine and clomipramine, are inactive to block stress-induced hyperthermia. The specific serotonin reuptake inhibitor, fluvoxamine, partly, not dose dependently and non-specifically antagonized stress-induced hyperthermia due to an increased basal T. The serotonin reuptake enhancer, tianeptine (Mennini et al., 1987), and the catecholamine reuptake inhibitor, nomifensine, also non-specifically antagonized stress-induced hyperthermia due to an increased basal T. The 5-HT releaser, *d,l*-fenfluramine, also non-specifically antagonized stress-induced hyperthermia due to an increased basal T. 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. It can be argued that the mice should have been pretreated chronically with antidepressants, because it is known that these compounds are clinically active only after 2–3 weeks' treatment. So far we have not tested this option.

Several other drugs acting via stimulation or block of serotonin receptors were also tested in this model. The $5\text{-HT}_{2A/C}$ receptor agonist, DOI (Olivier et al., 1993), blocked stress-induced hyperthermia due to a non-dose-dependent increase of basal T, also found by Salmi et al. (1994), which we interpret as a non-specific effect. The mixed $5\text{-HT}_{1A/B}$ receptor agonist, eltoprazine (Olivier et al., 1994b), does not block stress-induced hyperthermia significantly. The $5\text{-HT}_{1B/2C}$ receptor agonist, mCPP, antagonized stress-induced hyperthermia at the highest dose tested (10 mg/kg p.o.) probably due to the non-significant increase in basal T. The $5\text{-HT}_{2A/C}$ receptor antagonist, ketanserin, did not block stress-induced hyperthermia although a large decrease in basal T was observed. The results for ketanserin show that stress-induced hyperthermia may occur independently of a decrease in basal T. The 5-HT_3 receptor antagonist, ondansetron, which is known to possess anxiolytic activity in some anxiety tests (Olivier et al., 1992; Costall et al., 1989a,b) was inactive to prevent stress-induced hyperthermia.

Our results are generally highly consistent with those of Lecci et al. (1990a,b, 1991)) and show 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, are inactive to block stress-induced hyperthermia. These results are consistent with those of Lecci et al. (1990a,b)) who 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 of a decrease in basal T.

The antipyrogenic, acetyl salicylic acid, induced a significant increase in basal T resulting in a non-specifically decreased ΔT. Lecci et al. (1990a) reported no effect on basal T and end T and also concluded that mechanisms other than those involved in fever are involved in stress-induced hyperthermia, because if the hypothermia is caused by fever, one should expect the release of prostaglandins (Feldberg and Milton, 1978) and hence antagonism of the hypothermia by antipyretics like acetyl salicylic acid (Singer et al., 1986).

The catecholamine releaser, *d,l*-amphetamine, gave peculiar results as mentioned earlier. At 3 mg/kg i.p. basal T was decreased while at 10 mg/kg basal T was increased. However, ΔT was non-specifically decreased only at 10 mg/kg. The results of Lecci et al. (1990a) also showed an increase in basal T at 10 mg/kg but surprisingly end T was also significantly increased, even above the end T of the control mice. Apparently, in the Lecci et al. (1990a) study the end T was not maximal, whereas our results point strongly to a fixed maximal end T. We do not have an explanation for this discrepancy. However, amphetamine did not show specific anxiolytic properties in either of the stress-induced hyperthermia models.

The antihypertensive drugs, clonidine (α_2-adrenoceptor agonist) and prazosine (α_1-adrenoceptor antagonist), did prevent stress-induced hyperthermia but also produced a decreased basal T at all doses tested. The ΔT was significantly reduced only at the highest dose tested. The results for prazosine were a replication of those obtained by Lecci et al. (1990a) who suggested that the anxiolytic effects of prazosine can be explained by its centrally mediated antihypertensive effects and are not due to their temperature-lowering effects.

The central CCK_B 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 non-competitive 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, 0.3 mg/kg p.o., basal T was increased significantly. The end T was increased 0.36°C above the vehicle end T, so that ΔT was not significantly reduced. These results are consistent with findings of Lecci et al. (1991). However these authors also tested a higher dose, 1 mg/kg p.o., of MK 801, and reported an increased basal T and, surprisingly, a decreased end T resulting in a decreased ΔT. We have also tried 1 mg/kg MK 801 but because convulsions occurred no data could be obtained.

In conclusion, results obtained with our stress-induced hyperthermia model with male group-housed mice showed a more or less fixed maximal end T (physiological limit) which implies 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 to be anxiolytic. An advantage of this relative simple and robust stress-induced hyperthermia model is the fact that it can be used without test compounds interfering with feeding, drinking and nociception. This model also does not require time-consuming training procedures, and thus offers certain benefits for detecting anxiolytic drugs.

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