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Stress-induced hyperthermia in mice: effects of flesinoxan on heart rate and body temperature

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Abstract

Stress-induced hyperthermia in mice has predictive validity for anxiolytic properties of drugs. In this paradigm, 60 min after drug administration rectal temperature is measured, which causes hyperthermia of 1–1.5°C (ΔT) in about 10 min. Flesinoxan, a selective 5-HT_{1A} receptor agonist with anxiolytic-like properties, causes hypothermia, which complicates interpretation of stress-induced hyperthermia. Therefore, we combined flesinoxan treatment and the stress paradigm with radiotelemetric measurement of body temperature and heart rate, which is also related to anxiety. Subjects were either undisturbed or injected with flesinoxan (0–0.1–0.3–1.0 and 3.0 mg/kg), with or without the stress paradigm. Flesinoxan (1.0 and 3.0 mg/kg) caused a relatively long-lasting hypothermia, but did not lower heart rate. The rectal temperature procedure caused hyperthermia and tachycardia. Flesinoxan reduced the stress-induced hyperthermia and the tachycardia evoked by the stress procedure. Continuous radiotelemetric measurement of heart rate, apart from body temperature, revealed that flesinoxan has anxiolytic-like properties in mice.

Keywords: Anxiety; Stress; 5-HT_{1A} receptor; Tachycardia; Hyperthermia; Radiotelemetry

1. Introduction

Serotonin is a neurotransmitter involved in anxiety and depression (Van Praag, 1994; Roth, 1994; Leonard, 1996; Nemeroff, 1998). The complex role of serotonin in the underlying mechanisms is partly due to the plethora of receptors in the 5-HT system of which the 5-HT_{1A} receptor seems to play a pivotal role (for review: De Vry, 1995). 5-HT_{1A} receptor agonists have anxiolytic-like properties in many behavioral paradigms in animals (Korte and Bohus, 1990; Groenink et al., 1995a; Remy et al., 1996; Molewijk et al., 1996; Stanhope and Dourish, 1996; Sanchez, 1996; Xu et al., 1997) and in humans (O'Hanlon, 1991; Ratey et al., 1991). In one of these animal paradigms, stress-induced hyperthermia in mice (Borsini et al., 1989; Van der Heyden et al., 1997), anxiolytic-like properties of 5-HT_{1A} receptor agonists are reflected by their antagonism of the temperature rise induced by rectal temperature measurement (Lecci et al., 1990a,b; Zethof et al., 1995; Groenink et al., 1995b; Van der Heyden et al., 1997; Olivier et al., 1998). Rectal temperature is measured twice with a 10-min interval (T_1 and T_2 respectively) and drug injections are always given 60 min before the first measurement (T_1), in order to minimize an injection-induced effect (Van der Heyden et al., 1997). In general, body temperature changes are very prominent in a number of anxiety states and disorders (Lesch, 1991; Murphy et al., 1991), which lends support for face and construct validity of the stress-induced hyperthermia paradigm for human anxiety disorders (Van der Heyden et al., 1997). Moreover, 5-HT_{1A} receptor agonists, like flesinoxan, 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), buspirone and ipsapirone, induce hypothermia in animals (Goodwin et al., 1985, 1987; Hjorth, 1985; Hutson et al., 1987; Matsuda et al., 1990; Martin et al., 1992) and humans (Lesch et al., 1990; De Koning and De Vries, 1995; Seletti et al., 1995; Gelfin et al., 1995; Shiah et al., 1998). In this study, we combined flesinoxan treatment and the stress-induced hyperthermia

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paradigm with radiotelemetry for several reasons. The apparent contradiction between drug-induced hypothermia and blockade of the stress-induced hyperthermia is basically complicating the stress-induced hyperthermia procedure because body temperature is used as the dependent variable (Zethof et al., 1998). Radiotelemetry allows to sample body temperature continuously in freely moving mice, which gives us more insight in direct effects of flesinoxan treatment per se vs. putative anxiolytic properties in the stress paradigm. The stress of repeated handling, which directly affects body temperature, is avoided too. Moreover, by radiotelemetry a second parameter can be measured that is also associated with anxiety and stress, i.e. heart rate (Southwick et al., 1999). Stress-induced tachycardia can be reduced by activation of 5-HT_{1A} receptors (Korte and Bohus, 1990; Olivier et al., 1997). The bradycardia observed after 5-HT_{1A} receptor activation (Wouters et al., 1988; Dreteler et al., 1989; Hof and Fozard, 1989; Buisson et al., 1993; Chamienia and Johns, 1994a,b) seems to be due to an interaction with anaesthesia (Dreteler et al., 1990; Grohs et al., 1990), in that other studies using freely moving animals found opposite effects (Dedeoglu and Fisher, 1994; Di Francesco, 1994). Within our experimental setup, the effects of flesinoxan on heart rate, which shows fast responses to stimuli, were tested in mice. Because of the development of genetically modified mice, this species becomes more important in behavioral and physiological experiments. In this study, we attempt to elucidate the relation between direct effects of 5-HT_{1A} receptor activation on both body temperature and heart rate and putative anxiolytic-like properties in the stress-induced hyperthermia paradigm by using continuous radiotelemetric sampling.

2. Materials and methods

2.1. Subjects and maintenance

A group of 12 male mice (129/Sv–Ola strain) weighing 25.7 ± 0.4 g was obtained from the Central Animal Facility, Utrecht University, Utrecht, The Netherlands. Mice were socially housed until surgery, after which they were housed singly in Macrolon® Type 2 cages ($22 \times 16 \times 14$ cm) with continuous access to standard food pellets and tap water. Animals were kept under a controlled light/dark schedule (lights on between 7:00 am and 7:00 pm). Cages were enriched with nesting material (Enviro Dri®, BMI, Helmond, The Netherlands) and a gray opaque plastic tube (length 13.5 cm; ∅ 5 cm) to provide shelter and climbing facilities. The entire experiment was completed by 10 mice, because one transmitter had a technical failure and one animal died between tests, which was unrelated to the experiment. Only data of those mice that completed the whole experiment were analyzed. The ethical committee of the Faculty of Pharmacy of Utrecht University approved the experiment.

2.2. Surgery

A radiotelemetry device (type: ETA-F20; Data Sciences International (DSI), St. Paul, MN, USA) was implanted in the abdominal cavity of each animal. Mice were treated with the antibiotic Baytrill® (2.5 % enrofloxacin, 0.025 ml/mouse s.c.; 20 min prior to anaesthesia). During implantation, mice were anaesthetized with a mixture of Hypnorm®, Dormicum® and sterile water (1:1:2 in a volume of 10 ml/kg body weight i.p.). Two leads were positioned dorsal and ventral to the heart to allow measurement of the biopotential across the heart. After surgery, mice were treated for 2 days, twice a day with the analgesic Temgesic® (0.3 mg/ml; 3.0 ml/kg body weight i.p.). A detailed study about the effects of transmitter weight (3.8 g) on behavior revealed that mice recovered completely within 2 weeks (Baumans et al., in press). Therefore, animals were allowed to recover for 2 weeks after surgery before any telemetric measurement started. The present experiment started 6 weeks after surgery and was preceded by a baseline study, in which undisturbed circadian rhythm was determined.

2.3. Data acquisition

The implanted transmitter can be switched on and off by a magnet and emits radiowaves, which are collected per mouse by a receiver (type: RLA1020; DSI, St. Paul, MN, USA) positioned under each cage. These data were sent to a computer and translated into body temperature, heart rate and activity values (software under OS/2® Warp Connect: Dataquest™ A.R.T.™, DSI, St. Paul, MN, USA). Because we only had six receivers, animals were split in two separate groups. Over a 12-week period both groups were tested once a week. During a test all three parameters were sampled with 1-min intervals.

2.4. Experimental protocol

Data sampling started the afternoon prior to the test. Body weight was measured each week when transmitters were switched on. A slight increase in body weight was found during the entire study, while no effect of flesinoxan or repeated stress (stress-induced hyperthermia paradigm) was found. Each individual was treated 12 times with 1-week intervals. We only tested animals once a week to avoid interaction effects with previous treatments for pharmacological (drug washout) as well as methodological reasons (daily testing increases basal body temperature, Van der Heyden et al., 1997). In this study, a first comparison was made between animals not being treated (no injection) versus saline (0.9% NaCl) injection, to determine the effect of the injection procedure. The saline treatment was also compared with flesinoxan (0.1, 0.3, 1.0 and 3.0 mg/kg s.c.). These six treatments were either tested with or without the stress-induced hyperthermia

paradigm. Order of treatments over weeks as well as order of mice per test day was completely balanced over the entire study. Mice were injected using 2-min intervals. Injections started at 8:30 am, while rectal temperature measurements started at 9:30 am. The experimenter entered the test room exactly 5 min prior to the first injection, and left the test room during the period between injections and stress exposure. To determine putative indirect effects of flesinoxan treatment or the stress-induced hyperthermia procedure, sampling lasted for 30 h after the test (until 4:00 pm the next day). However, these data revealed that flesinoxan treatment or stress-induced hyperthermia exposure did not have any lasting or rebound effect on the parameters measured (data not shown).

2.5. Stress-induced hyperthermia paradigm in singly housed mice

Flesinoxan was administered 60 min prior to stress exposure. The stressor consists of measuring temperature (T_1) with a rectal probe (Digital Thermometer, Type 871A, Tegam, Geneva, OH, USA). This mild stressor causes an increase in temperature of about 1–1.5°C in 10 min. Therefore, 10 min later, the stressed level (T_2) is determined. The stress-induced hyperthermia, as a measure for anxiety, is determined as the difference between those temperatures ($\Delta T = T_2 - T_1$).

2.6. Drug

Flesinoxan-hydrochloride was synthesized by Solvay Pharmaceuticals, Weesp, The Netherlands. Each day, flesinoxan was freshly dissolved in saline and administered subcutaneously in the neck region, in a volume of 10 ml/kg body weight.

2.7. Data analysis

Mean group values ± S.E.M. of rectal temperatures were calculated and plotted (Fig. 1) for those treatments that involved the stress-induced hyperthermia paradigm. Radiotelemetric data were first averaged over five successive 1-min samples for each treatment and parameter, after which mean group values ± S.E.M. were calculated and plotted (Fig. 2). Data obtained during the particular minute of injection and rectal temperature measurements were skipped, because such handling procedures increased the transmitter–receiver distance resulting in loss of data. Because of the relatively small number of subjects compared to the vast amount of data collected, treatments were split into two groups for statistical analysis: treatments with and without stress-induced hyperthermia paradigm. Both groups contained the following treatments: no injection, saline, 0.1, 0.3, 1.0 and 3.0 mg/kg flesinoxan. The first two treatments were compared to determine injection effects per se. Effects of flesinoxan were analyzed across the dose–response curve (saline plus four doses).

Data were analyzed over specific time windows, chosen in relation to the manipulations. To investigate injection/flesinoxan effects, the average baseline value over a 30-min time window prior to injection (Fig. 2, *X*-axis: $-90 \sim -60$ min) was compared with the average value after injection ($-30 \sim 0$ min). These time windows were chosen in relation to drug absorption and distribution, as well as the time-point of stress exposure. For treatments with stress exposure (Fig. 2B,D,F), the effect of both stressors (T_1 and T_2) was analyzed across baseline values prior to stress (*X*-axis: $-5 \sim 0$ min) and values after stress. Responses of heart rate and activity are fast and immediate, while body temperature reacts somewhat slower. Therefore, responses of heart rate and activity were analyzed over the first 5 min immediately after each stressor (*X*-axis: $0 \sim 5$ and $10 \sim 15$ min, respectively), while body temperature was analyzed with a 5-min delay (*X*-axis: $6 \sim 10$ and $16 \sim 20$ min). Data were analyzed by univariate repeated measures analysis of variance (ANOVA) with Greenhouse–Geisser epsilon (ε) correction for putative violation of the sphericity assumption (Vasey and Thayer, 1987). Manipulations (injection/drug and stress-induced hyperthermia) were analyzed as a

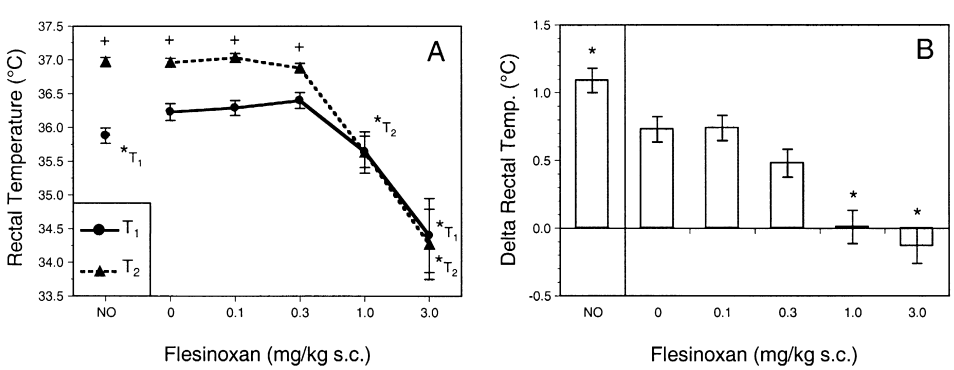

Fig. 1. Rectal temperature of singly housed male mice ($n = 10$) in the stress-induced hyperthermia paradigm, without treatment (NO) or after saline (0) and flesinoxan injection (0.1–3.0 mg/kg s.c.). Rectal temperature was measured twice (T_1 and T_2) with an interval of 10 min. T_1 (filled circles; drawn line) is the basal value, T_2 (filled triangles; stippled line) represents the stressed levels (A). The stress-induced hyperthermia (B) is represented by ΔT ($T_2 - T_1$). Results are shown as mean ± S.E.M. * means $P < 0.05$ vs. to saline, + means $P < 0.05$ vs. T_1.

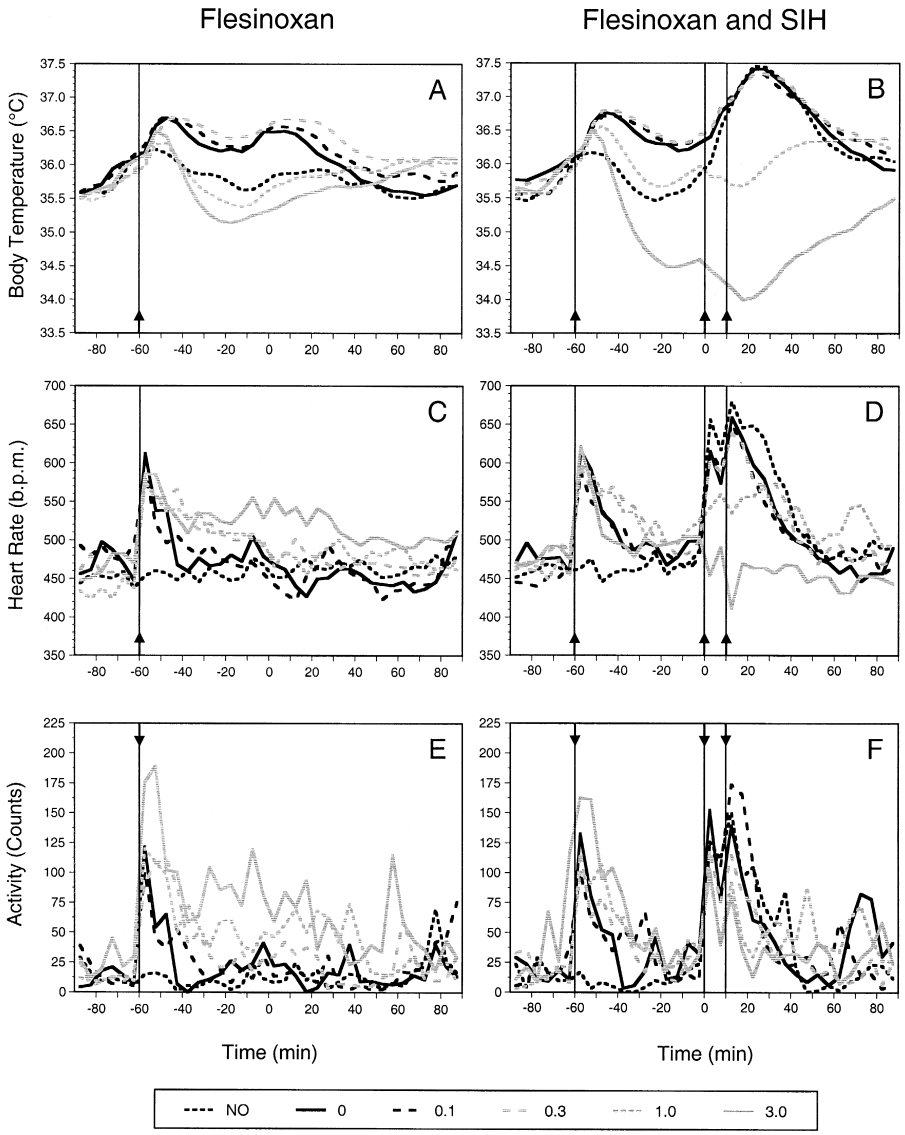

Fig. 2. Telemetrically determined body temperature (A,B), heart rate (C,D) and activity (E,F). Animals were not treated (NO: bold stippled, black line) or injected with saline (0 = drawn black line) and flesinoxan at −60 min (0.1 = stippled, black line; 0.3 = stippled, gray line; 1.0 = bold stippled, gray line; 3.0 mg/kg s.c. = drawn gray line). Time of injection (−60 min), T_1 (0 min) and T_2 (10 min) for the stress-induced hyperthermia (SIH) paradigm are indicated by vertical lines plus arrows. Data represent mean group values, averaged over 5-min periods. For clarity no S.E.M. is presented. To indicate the size of variance, mean values of S.E.M. over all 5-min blocks per panel are presented: panel A: ±0.18°C; panel B: ±0.17°C; panel C: ±18.4 b.p.m.; panel D: ±18.4 b.p.m.; panel E: ±13.5 Counts; panel F: ±15.7 Counts.

within-subject factor. Post-hoc comparisons were tested using *T*-tests with Bonferroni corrections for repeated comparisons. Significance was accepted at $P < 0.05$. All statistical analyses were performed using SPSS (Windows v.9.0, SPSS, Chicago, IL, USA).

3. Results

3.1. Rectal temperature: effects of flesinoxan in the stress-induced hyperthermia paradigm

Fig. 1 shows mean temperatures determined by the rectal probe, which are comparable to radiotelemetric data (cf. Fig. 2B). Saline injection affects the stress-induced hyperthermia (injection × stress: $F(1,9) = 5.54$, $P < 0.05$) as evidenced in a reduction in ΔT (Fig. 1B: NO vs. 0) resulting from a small elevation of 'baseline' temperature (T_1), 60 min after injection, but not in T_2 (Fig. 1A). Flesinoxan dose-dependently affected temperature (dose × stress: $F(4,36) = 18.36$, $P < 0.001$, $\varepsilon = 0.74$) by decreasing absolute levels (T_1 and T_2), but also reducing stress-induced hyperthermia (ΔT), leading to significant effects at 1.0 and 3.0 mg/kg.

3.2. Effects of flesinoxan on body temperature, heart rate and activity (radiotelemetry)

Direct effects (*X*-axis: −30 ~ 0 min) of saline/flesinoxan injection on body temperature (Fig. 2A,B),

heart rate (C,D) and activity (E,F) were determined in both groups of treatments.

While prior to injection temperature was stable, saline injection caused an increase, which was still significant 30–60 min later compared to untreated mice (injection × time: Fig. 2A; $F(1,9) = 9.01$, $P < 0.05$; Fig. 2B; $F(1,9) = 20.69$, $P < 0.005$). The small increases in body temperature, found between 60 and 90 min after injection (Fig. 2A), might be caused by witness stress when other mice were exposed to the stress-induced hyperthermia paradigm in the same test room (cf. Fig. 2B). Heart rate was increased immediately after saline injection (not analyzed), but returned to baseline levels within 30 min (Fig. 2C,D). Without injection, mice showed no effect on heart rate. Activity showed somewhat variable results; the significant interaction of injection and time (Fig. 2E; $F(1,9) = 5.09$, $P < 0.05$) could not be reproduced (Fig. 2F; $F(1,9) = 1.99$, $P = 0.19$). However, immediately after injection, activity increased briefly, while untreated mice showed no effect (not analyzed).

Flesinoxan dose-dependently induced hypothermia, which nearly reached significance in one group (dose × time: Fig. 2A; $F(4,36) = 4.07$, $P = 0.059$, $\varepsilon = 0.33$) and was highly significant in the other group of treatments (Fig. 2B; $F(4,36) = 8.26$, $P < 0.005$, $\varepsilon = 0.48$). Apart from non-specific immediate increases (not analyzed), 30–60 min after injection heart rate was unaffected by flesinoxan. Both groups of treatments showed a main effect of time (Fig. 2C; $F(1,9) = 5.82$, $P < 0.05$: Fig. 2D; $F(1,9) = 8.67$, $P < 0.05$), which suggests that, overall, heart rate was somewhat elevated after injection, independent of flesinoxan treatment. Activity revealed somewhat variable results again. The first group of treatments showed a significant interaction of dose and time (Fig. 2E; $F(4,36) = 4.78$, $P < 0.05$, $\varepsilon = 0.62$), which could not be replicated (Fig. 2F; $F(4,36) = 0.56$, $P = 0.53$, $\varepsilon = 0.35$).

3.3. Effects of flesinoxan on body temperature, heart rate and activity in the stress-induced hyperthermia paradigm (radiotelemetry)

Stress-induced hyperthermia, measured 10 min after the first rectal measurement, was reduced after injection compared untreated mice (NO) (injection × stress: Fig. 2B; $F(2,18) = 4.91$, $P < 0.05$). As described earlier, this effect is due to the elevated 'baseline' temperature prior to stress exposure (T_1) in saline-treated mice, while the stressed temperature (T_2) was similar to untreated mice. These radiotelemetric data are comparable to rectally determined values (Fig. 1A). The other two parameters, heart rate and activity, showed significant effects of stress only (Fig. 2D; $F(2,18) = 118.82$, $P < 0.001$, $\varepsilon = 0.92$; Fig. 2F; $F(2,18) = 44.59$, $P < 0.001$, $\varepsilon = 0.86$), indicating that the injection procedure had no residual effect on stress-induced tachycardia and hyperactivity. Flesinoxan (1.0 and 3.0 mg/kg) completely blocked the stress-induced hyperthermia (dose × stress: Fig. 2B; $F(8,72) = 24.32$, $P < 0.001$, $\varepsilon = 0.33$). Moreover, flesinoxan (1.0 and particularly 3.0 mg/kg) also blocked the stress-induced tachycardia (dose × stress: Fig. 2D; $F(8,72) = 16.72$, $P < 0.001$, $\varepsilon = 0.42$). Activity showed no significant interaction, but only a main effect of stress (Fig. 2F; $F(2,18) = 43.81$, $P < 0.001$, $\varepsilon = 0.86$) indicating that rectal temperature measurement caused hyperactivity, which was not affected by flesinoxan treatment.

4. Discussion

Flesinoxan affects temperature by causing hypothermia, as shown by direct rectal measurement (Fig. 1A: T_1) and radiotelemetry (Fig. 2A,B). Our data confirm previous studies in mice showing a dose-dependent decrease in body temperature after treatment with 5-HT_{1A} receptor agonists like 8-OH-DPAT (Goodwin et al., 1985; Matsuda et al., 1990; Lecci et al., 1990a; Martin et al., 1992; Young et al., 1994) and flesinoxan (Olivier et al., 1994, 1998; Groenink et al., 1996; Van der Heyden et al., 1997), which could be blocked by 5-HT_{1A} receptor antagonists (Fletcher et al., 1995; Forster et al., 1995; Patel and Hutson, 1996). However, in the present study flesinoxan-induced hypothermia was somewhat larger than reported in the other studies. One explanation could be a difference in housing conditions; we housed telemetered mice singly for weeks, while in the other studies mice were socially housed (Groenink et al., 1996) or singly for one day (Van der Heyden et al., 1997; Olivier et al., 1998). Alternative explanations concern the route of administration of the drug or strain of mice used. In contrast to body temperature, heart rate was not affected by flesinoxan. These findings contrast studies in anaesthetized subjects showing flesinoxan-induced bradycardia (Wouters et al., 1988; McCall et al., 1994; Chamienia and Johns, 1994a,b), probably emphasizing the importance of the sympathetic tone under freely moving conditions (Dreteler et al., 1990; Grohs et al., 1990). In humans, some studies on the partial 5-HT_{1A} receptor agonists buspirone and ipsapirone found a small decrease in heart rate (Unrug et al., 1997; Lechin et al., 1998), but others did not (Goa and Ward, 1986; Rammsayer et al., 1993). In general, data on activity were not conclusive, due to large variation between and within animals.

Radiotelemetry elucidated that the injection procedure reduced stress-induced hyperthermia (ΔT) due to a small increase of 'basal' temperature prior to rectal temperature measurements. However, maximal temperature levels reached after stress exposure were similar (around 37.5°C). A validation study (Van der Heyden et al., 1997) reported stress-induced hyperthermia after 5, 10 and 20 min, but not 30 and 60 min intervals between T_1 and T_2, which could be confirmed in our radiotelemetric setting. Moreover, the

second stressor (T_2) increased temperature further, which has been reported previously (Olivier et al., 1994; Van der Heyden et al., 1997). Flesinoxan (1.0 and 3.0 mg/kg) completely reduced the stress-induced hyperthermia (ΔT) confirming previous reports (Zethof et al., 1994; Groenink et al., 1995b, 1996; Van der Heyden et al., 1997; Olivier et al., 1997). Flesinoxan exerts these anxiolytic-like properties via 5-HT_{1A} receptors, because this could be antagonized by selective blockade of 5-HT_{1A} receptors (Olivier et al., 1998). The increase in heart rate, induced by the rectal temperature procedure, was similar in untreated and saline-injected mice. In contrast to body temperature, which remained elevated for about 60 min, tachycardia only lasted for about 40 min. This stress-induced tachycardia was reduced by 1.0 mg/kg, and completely blocked by 3.0 mg/kg flesinoxan. Therefore, the anxiolytic-like effect induced by 5-HT_{1A} receptor activation is not only reflected in the temperature, but also by the fast heart rate response. Locomotor activity was not affected significantly by flesinoxan, probably due to the size of variation. This radiotelemetric study elucidates that the saline injection procedure already affects baseline levels. Immediately after injection, heart rate showed an increase of 35% (from 450 to 620 b.p.m.). Such a strong tachycardia upon handling has been reported previously in mice and does not seem to habituate in this species (Stiedl and Spiess, 1997). This tachycardia was accompanied by increased activity and lasted approximately 20–30 min before returning to baseline levels. Thus, prior to stress-induced hyperthermia exposure, heart rate and activity were back at baseline levels. In contrast, body temperature displayed a delayed response and remained somewhat elevated 30–60 min after injection. In the standard, non-telemetric paradigm, the direct, flesinoxan-induced hypothermia complicates interpretation of inhibiting stress-induced hyperthermia. However, the present study elucidates that another important parameter involved in anxiety-related processes, i.e. heart rate, is not affected by flesinoxan itself, but the stress-induced tachycardia can be blocked by flesinoxan. Therefore, heart rate measurement during stress exposure adds important information about anxiolytic-like properties of compounds. Moreover, this study shows that radiotelemetry is a powerful technique to measure such stress-related processes, in particular in a species like the mouse that appears very sensitive to mild procedural effects and shows poor habituation. With the expanding amount of behavioral and physiological research in mice, as a result of the recent development of genetic mice models, radiotelemetry adds an important tool. The characteristic of long-term continuous sampling yields information about the influence of such handling and injection procedures per se and emphasizes the importance to determine 'real' and appropriate baseline levels. This way a relatively small group of mice ($n = 10$) can easily be studied in multi-challenge experiments as within-subject controls.

It can be concluded from this study that flesinoxan shows anxiolytic-like properties in a rather simple, but robust paradigm by blocking both the stress-induced hyperthermia as well as tachycardia.

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