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Anxiolytic effects of flesinoxan in the stress-induced hyperthermia paradigm in singly-housed mice are 5-HT_{1A} receptor mediated

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Abstract

In the stress-induced hyperthermia paradigm in singly-housed male mice, two sequential rectal temperature measurements reveal the basal temperature (T_1) and, 10 min later, an enhanced body temperature (T_2), due to the stress of the first rectal measurement. The difference $T_2 - T_1$ (ΔT) is the stress-induced hyperthermia and putatively reflects a stress-induced anxiogenic response. The full 5-HT_{1A} receptor agonist flesinoxan ((+)-enantiomer), its (−)-enantiomer and the racemic mixture reduced stress-induced hyperthermia effects, indicating putative anxiolytic properties. The ratio of their potencies to reduce stress-induced hyperthermia was similar to their potency in receptor binding affinities for 5-HT_{1A} receptors, supporting that the anti-hyperthermia effects are mediated by the 5-HT_{1A} receptor. This was further substantiated when the 5-HT_{1A} receptor antagonists WAY 100635 ((*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl) cyclo-hexane carboxamine trihydrochloride) and DU 125530 (2-[4-[4-(7-chloro-2,3-dihydro-1,4-benzodioxin-5-yl)-1-piperazinyl]butyl]-1,2-benzisothiazol-3(2H)-one-1,1-dioxide, monomesylate) both were able to antagonize the anti-stress-induced hyperthermia effects of flesinoxan. The stress-induced hyperthermia paradigm in singly-housed mice represents a simple and robust paradigm to measure putative anxiolytic effects of drugs. © 1998 Elsevier Science B.V.

Keywords: 5-HT_{1A} receptor; Flesinoxan, enantiomer; WAY 100635; DU 125530; Hyperthermia, stress-induced; (Mouse)

1. Introduction

Stress-induced hyperthermia in singly-housed male mice appears to be a robust, reproducible and easy paradigm to study putative anxiolytic effects of drugs (Van der Heyden et al., 1997). In this paradigm individually-housed mice are subject to two sequential rectal temperature measurements with a 10 min interval. The first measurement is the basal temperature (T_1), the second one the (stress-)enhanced temperature (T_2) and the difference (ΔT) is the stress-induced hyperthermia. The anxiolytics diazepam and flesinoxan both reduced ΔT (Van der Heyden et al., 1997), reflecting putative anxiolytic effects in this paradigm. In contrast, the antidepressant amitriptyline had no effect, suggesting that the paradigm reflects anxiolytic specificity.

In a closely related paradigm, using group-housed stress-induced hyperthermia in mice (Zethof et al., 1994), we explored a broad variety of drugs (Zethof et al., 1995) including benzodiazepines, 5-HT_{1A} receptor agonists and various antidepressants and presented evidence that this experimental procedure is able to predict anxiolytic activity in psychoactive drugs. However, the number of mice needed to perform the group-housed stress-induced hyperthermia is extremely high and we (Van der Heyden et al., 1997) were able to develop a closely related paradigm in singly-housed animals, using only 10% of the original number of animals. Although the mechanism by which the stress-induced hyperthermia may be caused is different in group-housed (sequential, but only one measurement) and singly-housed mice (two sequential measurements), the stress-induced phenomenon appears similar (for an extensive discussion see Van der Heyden et al., 1997).

In the present study the effects of the 5-HT_{1A} receptor agonist flesinoxan in this singly-housed stress-induced hyperthermia paradigm is investigated more closely. Although it seems likely that the anti-hyperthermia effect of flesinoxan in the 'singly-housed stress-induced hyperthermia' paradigm is caused by its 5-HT_{1A} receptor agonistic

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action, further pharmacological evidence for the latter may be supported by two lines of research.

First, flesinoxan is a (+)-enantiomer which has a higher affinity ($K_i = 1.7$ nM) for the 5-HT_{1A} receptor than the (−)-enantiomer ($K_i = 15$ nM) or the racemic mixture ($K_i = 4.7$ nM). This in vitro-difference (Wouters et al., 1988) was also found in vivo, e.g. in drug-discrimination studies (Gommans et al., 1995). The present study investigates whether the different enantiomers and the racemic mixture have differential potencies in inhibiting singly-housed stress-induced hyperthermia.

Second, antagonism of the anti-hyperthermic effects of this 5-HT_{1A} receptor agonist by selective 5-HT_{1A} receptor antagonists, would further support the evidence for mediation of the anxiolytic effects by the 5-HT_{1A} receptor. Therefore, two 5-HT_{1A} receptor antagonists, DU 125530 (2-[4-[4-(7-chloro-2,3-dihydro-1,4-benzodioxin-5-yl)-1-piperazinyl]butyl]-1,2-benziso-thiazol-3(2H)-one-1,1-dioxide, monomesylate) and WAY 100635 ((*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-*N*-(2-pyridinyl) cyclohexane carboxamine trihydrochloride) were used to antagonize the anti-hyperthermic effects of flesinoxan. DU 125530 is a recently synthesized 5-HT_{1A} receptor antagonist with a reasonable selectivity for the 5-HT_{1A} receptor ($K_i = 0.7$ nM for the 5-HT_{1A} receptor; $K_i = 5.2$ nM for dopamine D_2 receptor; $K_i = 6.4$ nM for α_1-adrenoceptor; $K_i = 11$ nM for the dopamine D_3 receptor). In vitro and in vivo pharmacological studies have profiled both DU 125530 (Mos et al., 1997) and WAY 100635 (Fletcher et al., 1996) as potent and selective 5-HT_{1A} receptor antagonists.

2. Materials and methods

2.1. Animals

Male NMRI mice (Charles River, Sulzfeld, Germany) weighing approximately 12–14 g upon arrival in the laboratory were housed in groups of 10 animals per cage ($34 \times 22 \times 15$ cm) under non-reversed 12 h light–12 h dark cycle conditions (lights on from 07.00 to 19.00 h). The animals were housed at constant room temperature (21 ± 2°C) and relative humidity (60 ± 10%) with food and water freely available. On the day before an experiment mice were individually housed in smaller cages ($12 \times 18 \times 13$ cm). Experiments were carried out in laboratory-adapted animals between 9 a.m. and 3 p.m. at least one week after their arrival.

2.2. General procedure

Cages were randomly and evenly allocated over daytimes (morning–afternoon) and treatments in each experiment. The temperature of mice was measured by inserting a thermistor probe for a length of 2 cm into the rectum of the mice. Digital recordings of the temperature were obtained with an accuracy of 0.1°C using a Keithley 871A digital thermometer (NiCr–NiAl thermocouple). The probe, dipped into silicon oil before inserting, was held in the rectum till a stable rectal temperature had been obtained for 20 s.

The animals were injected orally or subcutaneously with either a drug or vehicle (tragacanth suspension, 1% w/v) 60 min before the first temperature measurement (T_1). The temperature was again measured 10 min later (T_2).

In antagonism studies the antagonist and agonist were injected 60 min before the first rectal temperature measurement, one immediately before the other.

2.3. Statistics

For each individual mouse a basal temperature (T_1), an end temperature (T_2) and the difference (ΔT) = $T_2 - T_1$ was determined.

After homogeneity of variance for the variables T_1, T_2 and ΔT was checked between treatment, treatment effects were evaluated using a two-way analysis of variance, with explanatory factors treatment and daytime, together with all the interactions.

If the overall analysis of variance appeared significant, post-hoc *t*-tests were used to identify significant differences.

3. Results

Fig. 1A shows the effects of the (+)-enantiomer of flesinoxan on singly-housed stress-induced hyperthermia. Analysis of variance showed no significant overall effect of (+)-flesinoxan on T_1 ($F(6, 137) = 1.55$; $P = 0.16$) but a significant overall effect on T_2 ($F(6, 137) = 18.94$; $P < 0.0001$). Post-hoc comparisons showed that 10 mg/kg flesinoxan significantly decreased T_1, whereas T_2 was already significantly decreased at 1 mg/kg and higher doses. ΔT was significantly decreased at doses from 0.3 mg/kg and higher ($F(6, 137) = 18.94$; $P < 0.0001$).

The (−)-enantiomer of flesinoxan also decreased T_1 ($F(4, 63) = 2.94$; $P = 0.027$), having a significant effect at 1, 10 and 30 mg/kg p.o. T_2 is also decreased by (−)-flesinoxan ($F(4, 63) = 16.22$; $P < 0.0001$), leading to significant decreases from 10 mg/kg onwards. ΔT was significantly decreased from 3 mg/kg p.o. onwards ($F(4, 63) = 8.88$; $P < 0.001$).

The racemic mixture had both a significant effect on T_1 ($F(4, 63) = 2.94$; $P = 0.027$) and T_2 ($F(4, 63) = 15.13$; $P < 0.0001$) leading to significant decreases at 3 mg/kg (T_1) and 1 mg/kg (T_2), respectively. ΔT was significantly decreased ($F(4, 63) = 12.53$; $P < 0.0001$) at 1 mg/kg p.o. and higher doses.

For antagonism studies one dose of (+)-flesinoxan (3 mg/kg p.o.) was selected.

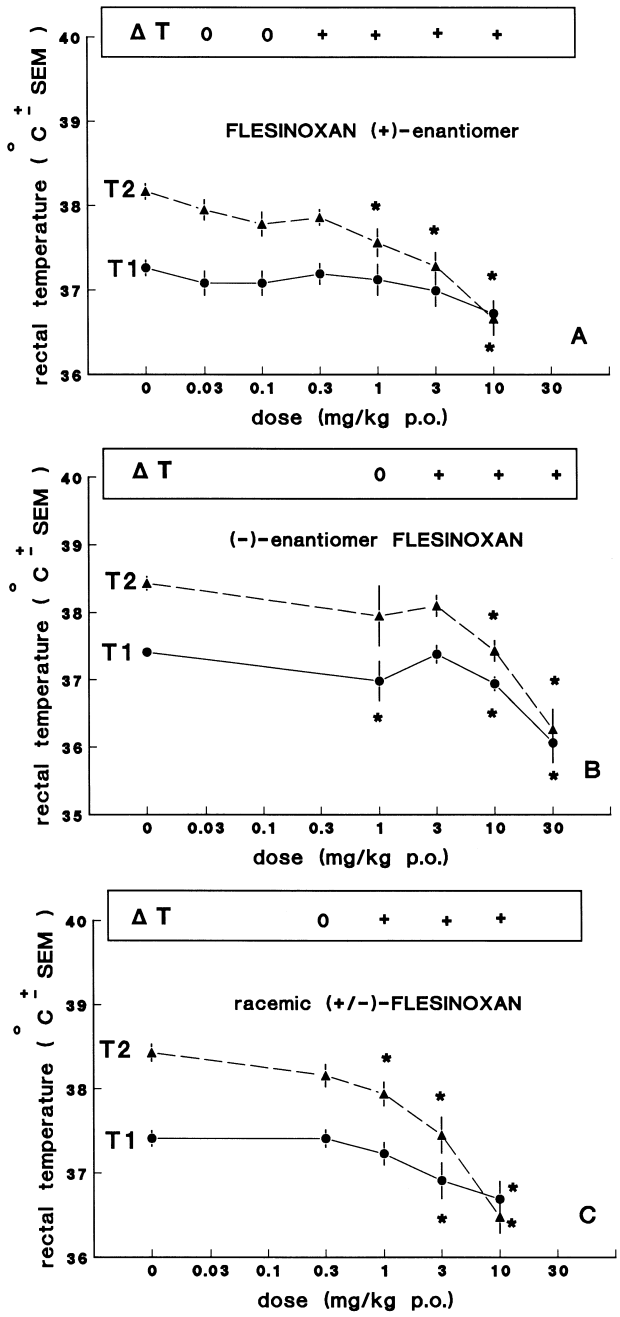

Fig. 1. The effects of flesinoxan ((+)-enantiomer) (A), (−)-flesinoxan (B) and (±)-flesinoxan (C) on stress-induced hyperthermia in singly-housed mice. Mice ($N = 8$ per dose) are measured twice, T_1 and T_2 with an interval of 10 min. $\Delta T (= T_2 - T_1)$ is indicated in the bar above each figure. Injections are administered 60 min before T_1. $^{*}P < 0.05$ indicates a significant difference from the corresponding vehicle (0 mg/kg) dose. $^{+}P < 0.05$ indicates a significant difference in ΔT after vehicle (0 mg/kg) treatment; 0 indicates no significant effects.

Fig. 2 shows the results of this dose or vehicle together with the 5-HT_{1A} receptor antagonist WAY 100635 (0.3, 1 or 3 mg/kg, s.c.).

Fig. 2A shows that pretreatment of WAY 100635 with vehicle treatment has neither any effect on T_1 ($F(3, 29) = 1.13$; $P = 0.35$), on T_2 ($F(3, 29) = 0.36$; $P = 0.78$), nor on ΔT ($F(3, 29) = 0.82$; $P = 0.49$).

Fig. 2B shows that pretreatment of WAY 100635 with flesinoxan treatment (3 mg/kg p.o.) leads to significant antagonism of flesinoxan's effect. Although the effect of flesinoxan on T_1 ($F(3, 26) = 2.26$; $P = 0.10$) was marginally antagonized by WAY 100635, that on T_2 ($F(3,$

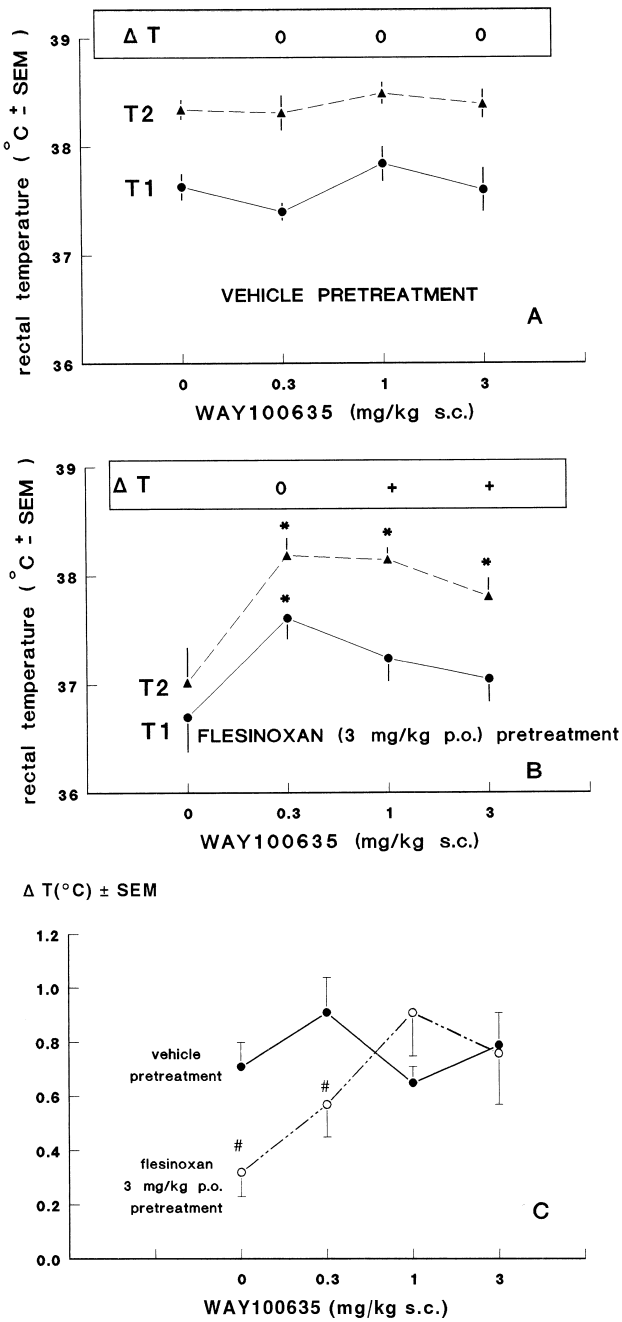

Fig. 2. The effects of vehicle-pretreatment (A), or flesinoxan-pretreatment (3 mg/kg, p.o.) (B) together with a dose-range treatment of WAY 100635 (0, 0.3, 1 and 3 mg/kg s.c.) is shown on T_1, T_2 and ΔT in stress-induced hypothermia in singly-housed mice ($N = 8$ per dose). (C) shows the corresponding ΔT's. $^{*}P < 0.05$ indicates a significant difference compared to the corresponding vehicle pretreatment. $^{\#}P < 0.05$ in (C) indicates a significant difference in ΔT between vehicle-pretreated and flesinoxan pretreated animals at similar doses of WAY 100635. $^{+}P < 0.05$ indicates a significant difference in ΔT (bars in A and B) compared to ΔT at (0 + 0)-doses; whereas 0 means no significant change.

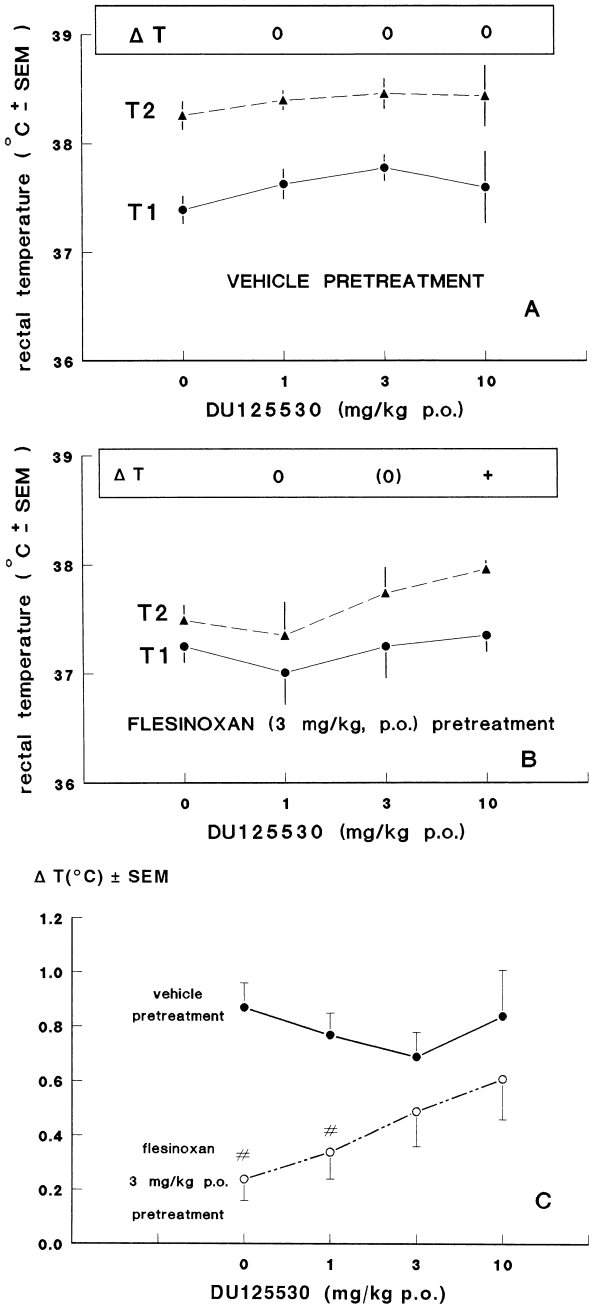

Fig. 3. The effects of vehicle-pretreatment (A), or flesinoxan-pretreatment (3 mg/kg, p.o.) (B) together with a dose-range treatment of DU 125530 (0, 1, 3 and 10 mg/kg, p.o.) is shown on T_1, T_2 and ΔT in stress-induced hyperthermia in singly-housed mice ($N = 8$ per dose). (C) shows the corresponding ΔT's. $^{\#}P < 0.05$ in (C) indicates a significant difference in ΔT between vehicle pretreatment and flesinoxan pretreatment at similar doses of DU 125530. $^{+}P < 0.05$ indicates a significant difference in ΔT (bars in A and B) compared to ΔT at (0 + 0)-doses; whereas 0 means no significant change.

26) = 4.98; $P = 0.007$) was clearly antagonized by WAY 100635, already at the lowest dose tested. Similarly, the decrease in ΔT caused by 3 mg/kg p.o. flesinoxan (Fig. 2C), was significantly antagonized ($F(3, 26) = 8.01$; $P = 0.001$) by the 1 and 3 mg/kg dose of WAY 100635.

Fig. 3 shows the results of a 3 mg/kg p.o. dose of flesinoxan or vehicle combined with the 5-HT_{1A} receptor antagonist DU 125530 (1, 3 or 10 mg/kg p.o.).

Fig. 3A shows that DU 125530 pretreatment after vehicle has neither an effect on T_1 ($F(3, 29) = 0.95$; $P = 0.43$), nor T_2 ($F(3, 29 = 0.40$; $P = 0.75$) or consequently on ΔT ($F(3, 29) = 0.57$; $P = 0.64$).

Fig. 3B shows that pretreatment with DU 125530 on flesinoxan's inhibitory effect (3 mg/kg p.o.) had neither significant effects on T_1 ($F(3, 29) = 0.33$; $P = 0.80$) nor influenced T_2 ($F(3, 29) = 1.66$; $P = 0.29$), but ΔT (Fig. 3C) was significantly affected ($F(3, 29) = 3.04$; $P = 0.04$), already (marginally) at 3 mg/kg ($P = 0.071$) and higher doses.

4. Discussion

Flesinoxan, the (+)-enantiomer, potently ($LED_{\Delta T} = 0.3$ mg/kg p.o.) reduced stress-induced hyperthermia (ΔT) in the singly-housed stress-induced hyperthermia paradigm, thereby confirming earlier findings (Van der Heyden et al., 1997). Both the (−)-enantiomer ($LED_{\Delta T} = 3$ mg/kg p.o.) and the racemic mixture ($LED_{\Delta T} = 1$ mg/kg p.o.) also exerted anti-hyperthermic effects. The ratio of their potency in the singly-housed stress-induced hyperthermia test is (+)-flesinoxan > (±)-flesinoxan > (−)-flesinoxan, which nicely corresponds to their affinity for the 5-HT_{1A} receptor (Table 1). This strongly supports the view that the 5-HT_{1A} receptor mediates the effects of flesinoxan in this stress-induced hyperthermia paradigm.

Thus, the anti-hyperthermia actions of flesinoxan and its antagonism by the selective 5-HT_{1A} receptor antagonists, WAY 100635 and DU 125530 strongly supports a role for the 5-HT_{1A} receptor as target receptor for putative anxiolytic drugs.

Flesinoxan, like other 5-HT_{1A} receptor agonists, has a broad anxiolytic profile in various animal models of anxiety, including conflict procedures in pigeons (Barrett et al., 1989; Barrett, 1992) and in rats (King et al., 1997), conditioned ultrasonic vocalizations in male rats (Sanchez, 1993; Molewijk et al., 1995a; Groenink et al., 1996a),

Table 1
The affinity of the two flesinoxan enantiomers and the racemic mixture (K_i in nM) for the 5-HT_{1A} receptor (human 5-HT_{1A} receptor expressed in CHO-cells) and their lowest effective dose (LED in mg/kg p.o.) in the singly-housed stress-induced hyperthermia-paradigm on T_1, T_2 and ΔT are shown

Flesinoxan-enantiomers	+	±	−
K_i (nM) 5-HT_{1A}[a]	1.7	4.7	15
LED_{T_1} (mg/kg)[b]	10	3	10
LED_{T_2} (mg/kg)	1	1	10
$LED_{\Delta T}$ (mg/kg)	0.3	1	3

[a] K_i-data are derived from Wouters et al. (1988).
[b] LED (= lowest effective dose) is the lowest dose tested showing a significant change compared to the representative control.

defensive burying in rats (Groenink et al., 1995), stress-induced hyperthermia in group-housed mice (Zethof et al., 1994; Groenink et al., 1996b), stretched approach posture in rats (Molewijk et al., 1995b), rat pup ultrasonic vocalizations (Olivier et al., 1994), guinea pig pup isolation calls (Molewijk et al., 1996), fear-potentiated startle in rats (Joordens et al., 1996) and in the elevated plus-maze in mice (Rodgers et al., 1994).

Moreover, flesinoxan induced a clear discriminatory stimulus in drug-discrimination procedures in rats (Ybema et al., 1990; Gommans et al., 1995) and pigeons (Mos et al., 1997). Like the effects of flesinoxan in the present study, the stimulus properties in drug discrimination studies of flesinoxan are clearly mediated by 5-HT_{1A} receptors, because the different enantiomers of flesinoxan had a similar ratio of potency as in the present study (Gommans et al., 1995). Moreover, the cue could also be antagonized by a 5-HT_{1A} receptor antagonist (WAY 100635).

A concern about the singly-housed stress-induced hyperthermia versus the group-housed version, is that the temperature rise induced by either method could be due to differential underlying mechanisms. In group-housed stress-induced hyperthermia, animals develop hyperthermia without being touched and handled, whereas in the singly-housed stress-induced hyperthermia an animal is handled and its rectal temperature is measured, before the second handling and rectal temperature measurement, 10 min later. It is already known for some time that insertion of a rectal thermometer increases body temperature (Poole and Stephenson, 1986). The first animals measured in the group-stress-induced hyperthermia, also develop hyperthermia (Zethof et al., 1994) but also the last animals, which remained relatively undisturbed except for the observation and disturbance of companion-animals picked up. Borsini et al. (1989) observed that if the number of animals in the group increased, and especially in the 20 animals/group/cage, the first 7 animals did not show hyperthermia. They conclude that the hyperthermia in groups is not only induced by physical stimuli (handling, movements) but that also anticipatory anxiety is involved. This anticipatory aspect is clearly not present in the singly-housed animal, where the stress is clearly linked to handling and rectal probe insertion. Although the cause of the stress hyperthermia seems different i.e. psychological versus physical, the mechanism behind the hyperthermia appears similar. Time-course (onset and end), induction of stress-hormones (Groenink et al., 1994; Bouwknecht et al., 1996) and pharmacological sensitivity (Zethof et al., 1995) are comparable in both paradigms. Although differences between stress-hyperthermia in group and singly-housed animals are present, e.g. in the size of ΔT (hyperthermia), these differences are probably more related to the process of being either isolated or in a group, than to differences in underlying mechanism. Whether the cause of the hyperthermia is only psychological or has also a physical part is not completely clear at this moment. It is possible that animals show motoric activation after the rectal temperature measurement, which on itself may lead to an enhanced body temperature. However, the psychological influence of the stressor must be important, because an enhanced body temperature is observed when animals have restricted space (unpublished findings). Moreover, administration of sedative drugs (e.g. ketanserin) which inhibit motor activity, still leave ΔT intact (Zethof et al., 1995), whereas flesinoxan at stimulatory doses (3–10 mg/kg) shows a decrease, instead of an increase in ΔT (this study).

It is a well-established fact that systemic administration of 5-HT_{1A} receptor agonists in mice (e.g. (±)-8-hydroxy-dipropylaminotetralin (8-OH-DPAT), ipsapirone, buspirone, gepirone and others) induces hypothermia (Goodwin and Green, 1985; Bill et al., 1991; Moser, 1991; Martin et al., 1992; Meller et al., 1992). Most of such studies use group-housed mice and the experimental procedures used are sometimes difficult to reconstruct exactly. Although appropriate controls are present, it is clear that all these studies do not take into consideration the fact that the procedures used, including handling and injections of the animals, induce considerable stress, which on itself leads to an increase in body temperature of approximately 1 to 1.5°C (Olivier et al., 1994; Van der Heyden et al., 1997). Under such circumstances, these studies therefore do not exclusively report the effect of a drug on basal body temperature, but rather on a not very well defined, but enhanced, body temperature. Therefore, the hypothermia reported after administration of 5-HT_{1A} receptor agonists (Wilkinson and Dourish, 1991) can be interpreted as a putative combination of intrinsic hypothermic effects and anxiolytic activity of the drug tested. Especially the fact that, after a stressor, one has to wait at least 60 min (Zethof et al., 1994; Van der Heyden et al., 1997; Olivier et al., 1994) before the body temperature has returned to basal, has not been taken into consideration. When using such a paradigm, the effect of a drug on the basal temperature (T_1), measured 60 min after the injection-stress, can be considered as a real drug effect on basal body temperature, whereas the measurement of the temperature 10 min later (T_2), reflects the effect of the drug on the stress-induced hyperthermia. Under such circumstances the effects of various 5-HT_{1A} receptor agonists (flesinoxan, buspirone, ipsapirone) do not, or only at high doses, decrease the basal temperature in group-housed stress-induced hyperthermia (Olivier et al., 1994; Zethof et al., 1995), or in the singly-housed stress-induced hyperthermia, like in the present study. There is some evidence (Olivier et al., 1994) that only an interaction of stress and 5-HT_{1A} receptor activation may lead to an effect on body temperature.

In conclusion, this study provides evidence for stress-induced transient hypoactivity of the serotonin system yielding stress-induced hyperthermia. This can be attenuated by the selective 5-HT_{1A} receptor antagonists. This complex interaction needs further investigation but implies the 5-HT_{1A} receptor in stress-mediated processes.

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References

Barrett, J.E., 1992. Studies on the effects of 5-HT_{1A} drugs in pigeons. Drug Dev. Res. 26, 299–317.

Barrett, J.E., Gleeson, S., Nader, M.A., Hoffman, S.M., 1989. Anticonflict effects of the 5-HT_{1A} compound flesinoxan. J. Psychopharmacol. 3, 64–69.

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. 103, 1857–1864.

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

Bouwknecht, J.A., Hijzen, T.H., Van der Gugten, J., Van der Heyden, J.A.M., Veening, J.G., Olivier, B., 1996. Stress-induced hyperthermia in individually housed mice: Hormonal correlates and fos-protein immunoreactivity. Neurobiology of stress. Elba Int. Neurosci. Program, 16.

Fletcher, A., Forster, E.A., Bill, D.J., Brown, G., Cliffe, I.A., Hartley, J.E., Jones, D.E., McLenachan, A., Stanhope, K.J., Critchley, D.J.P., Childs, K.J., Middlefell, V.C., Laufumey, L., Corradetti, R., Laporte, A.M., Gozlan, H., Hamon, M., Dourish, C.T., 1996. Electrophysiological, biochemical, neurohormonal and behavioural studies with WAY-100635, a potent, selective and silent 5-HT_{1A} receptor antagonist. Behav. Brain Res. 73, 337–353.

Gommans, J., Hijzen, T.H., Maes, R.A., Mos, J., Olivier, B., 1995. Discriminative stimulus properties of flesinoxan: Effects of enantiomers, (*S*)-UH301 and WAY 100635. Eur. J. Pharmacol. 284, 135–140.

Goodwin, G.M., Green, A.R., 1985. A behavioural and biochemical study in mice and rats of putative agonists and antagonists for 5-HT_1 and 5-HT_2 receptors. Br. J. Pharmacol. 84, 743–753.

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-HT1A receptor agonist, are not related to its neuroendocrine effects. Eur. J. Pharmacol. 280, 185–193.

Groenink, L., Van der Gugten, J., Olivier, B., 1996a. The 5-HT_{1A} receptor is not involved in emotional stress-induced rises in stress hormones. Pharmacol. Biochem. Behav. 55, 303–308.

Groenink, L., Van der Gugten, J., Van der Heyden, J.A.M., Zethof, T.J.J., Olivier, B., 1996b. Neuroendocrine effects of diazepam and flesinoxan in the stress-induced hyperthermia test in mice. Pharmacol. Biochem. Behav. 54, 249–254.

Joordens, R.J.E., Hijzen, T.H., Peeters, B.W.M.N., Olivier, B., 1996. Fear-potentiated startle response is remarkedly similar in two laboratories. Psychopharmacology 126, 104–109.

King, C.M.F., Gommans, J., Joordens, R.J.E., Hijzen, T.H., Maes, R.A.A., Olivier, B., 1997. Effects of 5-HT_{1A} receptor ligands in a modified Geller–Seifter conflict model in the rat. Eur. J. Pharmacol. 325, 121–128.

Martin, K.F., Phillips, I., Hearson, M., Prow, M.R., Heal, D.J., 1992. Characterization of 8-OH-DPAT-induced hypothermia in mice as a 5-HT_{1A} autoreceptor response and its evaluation as a model to selectively identify antidepressants. Br. J. Pharmacol. 107, 15–21.

Meller, E., Chalfin, M., Bohmaker, K., 1992. Serotonin 5-HT_{1A} receptor-mediated hypothermia in mice: Absence of spare receptors and rapid induction of tolerance. Pharmacol. Biochem. Behav. 43, 405–411.

Molewijk, H.E., Van der Poel, A.M., Mos, J., Van der Heyden, J.A.M., Olivier, B., 1995a. Conditioned ultrasonic distress vocalizations in adult male rats as a behavioural paradigm for screening anti-panic drugs. Psychopharmacology 117, 32–40.

Molewijk, H.E., Van der Poel, A.M., Olivier, B., 1995b. The ambivalent behaviour stretched approach posture in the rat as a paradigm to evaluate anxiolytic drugs. Psychopharmacology 121, 81–90.

Molewijk, H.E., Hartog, K., Van der Poel, A.M., Mos, J., Olivier, B., 1996. Reduction of guinea pig pup isolation calls by anxiolytic and antidepressant drugs. Psychopharmacology 128, 31–38.

Mos, J., Van Hest, A., Herremans, A.H.J., Van Drimmelen, M., Olivier, B., 1997. The putative 5-HT_{1A} receptor antagonist DU 125530 blocks the discriminative stimulus effects of the 5-HT_{1A} agonist flesinoxan in the pigeon. Eur. J. Pharmacol. 325, 145–153.

Moser, P.C., 1991. The effect of putative 5-HT_{1A} receptor antagonists on 8-OH-DPAT-induced hyperthermia in rats and mice. Eur. J. Pharmacol. 193, 165–172.

Olivier, B., Molewijk, E., Van Oorschot, R., Van der Poel, G., Zethof, T., Van der Heyden, J., Mos, J., 1994. New animal models of anxiety. Eur. Neuropsychopharmacol. 4, 93–102.

Poole, S., Stephenson, J.D., 1986. Core temperature: Shortcomings of rectal temperature measurements. Physiol. Behav. 18, 203–208.

Rodgers, R.J., Cole, J.C., Davies, A., 1994. Antianxiety and behavioral suppressant actions of the novel 5-HT_{1A} receptor agonist, flesinoxan. Pharmacol. Biochem. Behav. 48, 959–963.

Sanchez, C., 1993. Effect of serotonergic drugs on foot shock-induced ultrasonic vocalization in adult male rats. Behav. Pharmacol. 4, 269–277.

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.

Wilkinson, L.O., Dourish, C.T., 1991. Serotonin and animal behaviour. In: Peroutka, S.J. (Ed.), Serotonin Receptor Subtypes: Basic and Clinical Aspects. Wiley-Liss, New York, pp. 147–210.

Wouters, W., Tulp, M.Th.M., Bevan, P., 1988. Flesinoxan lowers blood pressure and heart rate in cats via 5-HT_{1A} receptors. Eur. J. Pharmacol. 149, 213–223.

Ybema, C.E., Slangen, J.L., Olivier, B., Mos, J., 1990. Discriminative stimulus properties of the serotonergic compound flesinoxan. Pharmacol. Biochem. Behav. 35, 781–784.

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.