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Behavioral and physiological mouse models for anxiety: effects of flesinoxan in 129S6/SvEvTac and C57BL/6J mice

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Abstract

Serotonin$_{1A}$ (5-HT$_{1A}$) receptors are involved in anxiety. This study focuses on the role of genetic factors on the anxiety-related effects of 5-HT$_{1A}$ receptor stimulation using both a within subject design. The effects of 5-HT$_{1A}$ receptor activation were studied in high- and low-anxiety mice (129S6/SvEvTac (S6) and C57BL/6J (B6), respectively) in behavioral and physiological anxiety-related assays. These two strains were also selected because they are frequently used in gene-targeting studies. Mice were treated with the selective 5-HT$_{1A}$ receptor agonist flesinoxan (0–0.3–1.0–3.0 mg/kg s.c.) and tested in either the open-field activity test, the light–dark exploration test, or the stress-induced hyperthermia paradigm. Flesinoxan unexpectedly increased anxiety, but also decreased activity on several behavioral measures in B6 mice. Flesinoxan produced only minimal effects in the behavioral tests in the high-anxiety S6 strain. In contrast, the physiological hyperthermia response showed anxiolytic-like effects of flesinoxan in both strains. Our data indicate that the role of 5-HT$_{1A}$ receptor activation on anxiety-related responses is dependent on genetic background and selected paradigm used to assess anxiety. These findings indicate that it is critical to use a multi-level approach to develop mouse models for human diseases. In addition, the implication of such findings for studies on genetically modified mice is discussed.

Keywords: Physiology; Behavior; Anxiety; Animal model; Flesinoxan; 5-HT$_{1A}$ receptor

1. Introduction

The development of gene targeting techniques in mice has caused a tremendous interest in studying various mouse models of human diseases. The creation of these mutant mouse models often results in a mutation on a mixed genetic background, of which the 129 substrains ×C57BL/6 background is the most common (Simpson et al., 1997). In order to avoid false positive results that might be due to the mixed background instead of the targeted mutation (Gerlai, 1996), it is important to collect detailed information about strain differences (Crawley et al., 1997). Based on behavioral paradigms like open-field activity, elevated plus and zero maze, and light–dark exploration, the 129 substrains are generally labeled as high-anxiety mice, whereas the C57BL/6 mice show low levels of anxiety (Bouwknecht and Paylor, 2002; Cook et al., 2002; McIlwain et al., 2001; Rodgers et al., 2002; Voikar et al., 2001). In addition, the level of locomotor activity is different in these two strains, in which C57BL/6 mice are far more active than 129 substrains (Logue et al., 1997; Paulus et al., 1999).

In the present study, we examined the effects of the 5-HT$_{1A}$ receptor agonist, flesinoxan on unconditioned anxiety responses in C57BL/6 and 129 mice. In this study, we attempt to discriminate behavioral from physiological responses, and anxiety-related from locomotor behavior. The two behavioral mouse assays studied are the light–

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dark exploration test (Costall et al., 1989; Crawley and Goodwin, 1980) and open-field activity paradigm (Christmas and Maxwell, 1970), which are both based on the aversion of mice for brightly lit environments. In the light–dark test, mice can avoid exposure to the illuminated area by entering a dark compartment, whereas mice in the brightly lit open-field box stay in close proximity with the walls (i.e. thigmotaxis) and avoid the center area. The light–dark and open-field paradigms provide a variety of behavioral indices for anxiety-related and locomotor responses. We also included a third physiological assay for anxiety, i.e. the stress-induced hyperthermia paradigm (Borsini et al., 1989; van der Heyden et al., 1997). The stress-induced hyperthermia paradigm measures an increase in body temperature after exposure to a mild stressor in a familiar environment. An important feature of the stress-induced hyperthermia paradigm is the fact that it is not influenced by locomotor activity.

We focus on the role of the 5-HT_{1A} receptor for a variety of reasons. The serotonergic system and in particular 5-HT_{1A} receptors have been associated with anxiety-related responses in both animal models and humans (see review Gingrich and Hen, 2001). 5-HT_{1A} receptor activation has been shown to reduce certain forms of anxiety-based responses in rodents (e.g. Groenink et al., 2000; King et al., 1997; Molewijk et al., 1996) and anxiety in patients (Eison and Eison, 1994; Feighner and Boyer, 1989). We selected flesinoxan as a selective 5-HT_{1A} receptor agonist (Wouters et al., 1988) with a longer half-life than 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) (Perry and Fuller, 1989; Zuideveld et al., 2002a,b), which makes it a suitable tool in order to evaluate the role of 5-HT_{1A} receptors in anxiety-related responses in mice.

Recent studies on 5-HT_{1A} receptor knockout mice highlighted the importance of genetic background and testing paradigm on anxiety-based responses (Groenink et al., 2003; Heisler et al., 1998; Olivier et al., 2001; Parks et al., 1998; Pattij et al., 2001; Ramboz et al., 1998). The present study generates within-subject dose–response curves for flesinoxan (0–0.3–1.0–3.0 mg/kg s.c.) in each of the three paradigms for the two commonly used background strains in gene targeting studies: i.e. 129S6/SvEvTac (S6) and C57BL/6J (B6) mice. In a recent paper, we described the consistency of strain differences in drug-free responses to the light–dark and stress-induced hyperthermia paradigm (Bouwknecht and Paylor, 2002). The prospective of testing the same individuals repeatedly is highly beneficial for multiple reasons, including the limited availability of mutant mice in many labs, and the statistical power of within-subject designs in drug treatment studies. Similar approaches have been previously applied, albeit to a limited extent (e.g. Blumstein and Crawley, 1983; Costall et al., 1989; Onaivi and Martin, 1989; Rodgers and Shepherd, 1993). The beneficial effect of treatment with a putative anxiolytic compound is thought to be most effective in very anxious mice. Therefore, we hypothesized that the anxiolytic effects of flesinoxan would be most significant across all paradigms in the high-anxiety 129S6/SvEvTac mice, whereas the effects in the low-anxiety C57BL6/J strain would be absent or limited.

2. Materials and methods

2.1. Subjects and maintenance

All experiments were performed at the Faculty of Pharmaceutical Sciences, Utrecht University, The Netherlands. C57BL/6J mice (B6: 30 males) were obtained from Jackson Laboratory, Bar Harbor, ME, USA and 129S6/SvEvTac mice (S6: 30 males) came from Taconic, Germantown, NY, USA. Mice were 8–9 weeks old at the start of the experiment. Prior to testing, mice were handled repeatedly and allowed to acclimate. Animals were housed socially per strain in Type 2 cages (22 × 16 × 14 cm; 5 mice per cage) with water and food available ad libitum. Lights were on between 6.00 a.m. and 6.00 p.m. Mice were housed and tested in accordance with NIH policies on use of animals in research. Both the Animal Protocol Review Committee at Baylor College of Medicine, Houston and the Animal Ethical Committee of the Faculty of Pharmaceutical Sciences of Utrecht University approved the testing procedures.

2.2. Experimental procedures

For details on the experimental procedures, we refer to a parallel study in which we determined the consistency of drug effects in the same three anxiety-related paradigms (Bouwknecht et al., 2004). Here, we only briefly describe each test. All animals were kept individually after injection during the early hours of the light phase (1-h injection-test interval). In the stress-induced hyperthermia paradigm, mice were singly housed the day before the test.

2.2.1. Open-field activity test

The 30-min open-field test is performed in an automated Plexiglas Digiscan optical animal activity system under 700–800 lx and 55-dB white noise. The system determines locomotor and vertical activity automatically using infrared beams dividing the box in a center (35% total surface) versus outer area.

2.2.2. Light–dark exploration test

The 10-min light–dark test is performed under 55-dB white noise in a polypropylene box (44 × 21 × 21 cm) divided in two chambers (1/3 dark, 2/3 brightly lit at 700–800 lx) (Crawley and Goodwin, 1980), which are connected through a small opening. The position of the

mouse inside the box was scored as well as rearing behavior and latency to enter the dark with The Observer® (Noldus Information Technologies, Leesburg, VA, USA: see (Bouwknecht and Paylor, 2002). In order to score a transition from one area to another, all four paws had to cross the line.

2.2.3. Stress-induced hyperthermia paradigm

The stress-induced hyperthermia paradigm reflects a physiological response to mild stress exposure (van der Heyden et al., 1997). On the test day, the probe (Thermalert TH-5, Physitemp, Clifton, NJ, USA) was dipped in oil and inserted in the rectum for about 2 cm (T_1: basal temperature). The mild stress of handling and probe insertion causes a hyperthermic response, which is determined 10 min later (T_2: stressed temperature). The difference ($\Delta T = T_2 - T_1$) reflects the stress-induced hyperthermia.

2.3. Experimental setup

The experiment was performed as a within-subject design, where each subject was tested four times at 1-week intervals in the open-field, light–dark, or stress-induced hyperthermia paradigm. For each paradigm and strain, 10 subjects were treated in a balanced order with one of four doses of flesinoxan (0.0–0.3–1.0–3.0 mg/kg s.c.) resulting in a complete dose–response curve for each subject.

2.4. Data analysis

In general, all parameters were analyzed as repeated measures analysis of variance (ANOVA: SPSS, Version 10 for Windows, Chicago, IL, USA). Strain was analyzed as a between-subjects factor, and dose as a within-subject factor. A Greenhouse–Geisser correction was used to correct for putative violations of the sphericity assumption (Vasey and Thayer, 1987). Both numerator and denominator degrees of freedom are multiplied by epsilon, and the significance of the F ratio evaluated with the new degrees of freedom. In addition to the initial analysis across both strains, dose–response curves were also analyzed for each strain separately. When appropriate, post-hoc comparisons were made with Bonferroni corrections to adjust for repeated comparisons.

For the open-field test, we focused on the following parameters: time and distance traversed in the center area, total distance in entire box, and the frequency of vertical movements. In addition, the center divided by total distance was calculated as an anxiety measure.

In the light–dark test, number of light–dark transitions, latency to enter and time spent in the dark area, and frequency of rearing were determined. For the stress-induced hyperthermia procedure, both basal temperature (T_1) and the hyperthermic response (ΔT) were analyzed as a repeated measure.

3. Results

3.1. Open-field activity test

The parameter used as an indicator for anxiety in the open-field test is center/total distance, which showed differences between the two inbred strains (see statistics in Table 1 and graphics in Fig. 1: top left panel). Moreover, there was a significant effect of flesinoxan dose. The interaction between strain and dose showed a trend. Flesinoxan decreased the relative activity in the center area in B6 mice, whereas the drug had no effect in S6 mice.

An alternative parameter for anxiety, which is less sensitive to strain differences in locomotor activity, is time spent in the center area (top right panel). The “low anxiety” B6 mice spent more time in the center area than S6 mice, whereas the effects of flesinoxan were strain-dependent. Flesinoxan dose-dependently reduced the time in the center area in B6 mice, but had no significant effect in S6 mice.

The total distance traversed in the open-field box (middle left panel) showed a significant interaction between dose and strain. Overall, B6 mice were more active in the open-field box than S6 mice, and the effect of flesinoxan was strain dependent. In B6 mice, flesinoxan caused a linear decrease in locomotor activity. In contrast, S6 mice showed a significant but nonlinear pattern in response to the drug; the lowest dose increased locomotion, whereas the highest dose showed the opposite effect.

The total distance traversed in the open-field arena is the sum of movement in the outer and the center area. The center area is more ‘anxiety-provoking’ for the mice and therefore analyzed separately (middle right panel). The effects of flesinoxan were strain-dependent. Similar to the total distance data, B6 mice were more active in the center area than S6 mice. B6 mice showed a dose-dependent decrease in their center activity. While flesinoxan induced a two-fold decrease in total locomotion in B6 mice, the distance traveled in the center was more severely affected by the drug (five-fold decrease). As a result, the center/total distance ratio as a measure for anxiety was reduced by flesinoxan in B6 mice (see above). In the center area, flesinoxan also reduced activity in S6 mice.

Finally, vertical activity reflects rearing behavior in the open-field box (bottom panel), which is a measure for exploratory behavior. The levels of vertical activity were different between the two strains. While S6 mice showed almost no rearing behavior, B6 mice were very active (e.g. vehicle: frequency is 10/min). The effect of flesinoxan was strain-dependent. Flesinoxan completely abolished vertical activity in B6 mice, but had no effect in S6 mice, which might be related to a floor effect.

3.2. Light–dark exploration test

In the light–dark box, B6 mice showed more light–dark transitions than the S6 strain (Fig. 2: top left panel), and the

Table 1
ANOVA results for each parameter in the open-field test, the light–dark test and the stress-induced hyperthermia (SIH) paradigm

Parameter (Test)	Dose (B6+S6 mice)	Strain	Dose × strain	Dose (B6 mice)	Dose (S6 mice)
Center/tot distance (Open-field)	$F_{3,54}=9.15$ $P<0.001$ $\varepsilon=0.92$	$F_{1,18}=40.84$ $P<0.001$	$F_{3,54}=2.52$ $P=0.074$ $\varepsilon=0.92$	$F_{3,27}=9.07$ $P<0.005$ $\varepsilon=0.70$	$F_{3,27}=1.83$ $P=0.18$ $\varepsilon=0.73$
Time in center (Open-field)	$F_{3,54}=4.77$ $P<0.01$ $\varepsilon=0.81$	$F_{1,18}=49.26$ $P<0.001$	$F_{3,54}=3.22$ $P<0.05$ $\varepsilon=0.81$	$F_{3,27}=4.98$ $P<0.005$ $\varepsilon=0.74$	$F_{3,27}=0.75$ $P=0.48$ $\varepsilon=0.60$
Total distance (Open-field)	$F_{3,54}=19.89$ $P<0.001$ $\varepsilon=0.80$	$F_{1,18}=27.57$ $P<0.001$	$F_{3,54}=6.45$ $P<0.005$ $\varepsilon=0.80$	$F_{3,27}=22.43$ $P<0.001$ $\varepsilon=0.82$	$F_{3,27}=7.69/$ $P=0.005$ $\varepsilon=0.62$
Center distance (Open-field)	$F_{3,54}=19.06$ $P<0.001$ $\varepsilon=0.67$	$F_{1,18}=27.14$ $P<0.001$	$F_{3,54}=10.22$ $P<0.001$ $\varepsilon=0.67$	$F_{3,27}=17.15$ $P<0.001$ $\varepsilon=0.57$	$F_{3,27}=3.70$ $P=0.05$ $\varepsilon=0.62$
Rearing (Open-field)	$F_{3,54}=40.65$ $P<0.001$ $\varepsilon=0.50$	$F_{1,18}=38.97$ $P<0.001$	$F_{3,54}=36.56$ $P<0.001$ $\varepsilon=0.50$	$F_{3,27}=39.03$ $P<0.001$ $\varepsilon=0.50$	$F_{3,27}=2.52$ $P=0.12$ $\varepsilon=0.59$
Light–dark transitions (Light–dark)	$F_{3,54}=12.45$ $P<0.001$ $\varepsilon=0.78$	$F_{1,18}=38.13$ $P<0.001$	$F_{3,54}=4.68$ $P<0.05$ $\varepsilon=0.78$	$F_{3,27}=9.20$ $P=0.001$ $\varepsilon=0.81$	$F_{3,27}=4.27$ $P<0.05$ $\varepsilon=0.51$
Time spent in dark area (Light–dark)	$F_{3,54}=2.13$ $P=0.12$ $\varepsilon=0.83$	$F_{1,18}=11.64$ $P<0.005$	$F_{3,54}=0.09$ $P=0.94$ $\varepsilon=0.83$	Not analyzed	Not analyzed
Time to enter dark area (Light–dark)	$F_{3,54}=4.85$ $P<0.01$ $\varepsilon=0.90$	$F_{1,18}=0.06$ $P=0.82$	$F_{3,54}=0.17$ $P=0.90$ $\varepsilon=0.90$	Not analyzed	Not analyzed
Rearing (Light–dark)	$F_{3,54}=11.04$ $P<0.001$ $\varepsilon=0.57$	$F_{1,18}=28.08$ $P<0.001$	$F_{3,54}=7.46$ $P<0.005$ $\varepsilon=0.57$	$F_{3,27}=9.62$ $P<0.005$ $\varepsilon=0.58$	$F_{3,27}=2.10$ $P=0.18$ $\varepsilon=0.36$
SIH amplitude (SIH)	$F_{3,54}=16.18$ $P<0.001$ $\varepsilon=0.86$	$F_{1,18}=24.03$ $P<0.001$	$F_{3,54}=0.35$ $P=0.76$ $\varepsilon=0.86$	Not analyzed	Not analyzed
Basal temperature (SIH)	$F_{3,54}=29.20$ $P<0.001$ $\varepsilon=0.86$	$F_{1,18}=12.34$ $P<0.005$	$F_{3,54}=4.97$ $P<0.01$ $\varepsilon=0.86$	$F_{3,27}=15.37$ $P<0.001$ $\varepsilon=0.85$	$F_{3,27}=17.96$ $P<0.001$ $\varepsilon=0.69$

In case of a significant interaction ($P<0.05$) or a trend ($P<0.10$) between dose and strain, the effects of dose were analyzed for each strain separately (last two columns). For detailed description of statistical analysis and overall effects, see Results.

effect of flesinoxan was strain-dependent. Flesinoxan dose-dependently reduced transition levels in both strains. However, post-hoc comparisons in S6 mice revealed no significant differences between vehicle and flesinoxan, which might be explained by their low activity levels.

Another parameter for anxiety-related responses in the light–dark test is the time spent in the dark area (Fig. 2, top right panel). In general, mice with higher levels of anxiety will spend more time in the dark area compared to mice with lower levels of 'anxiety'. B6 mice spent significantly less time (±40%) in the dark area than S6 mice (±70%), whereas neither the interaction, nor the main effect of flesinoxan was significant.

The light–dark test starts by placing a subject in the illuminated area against the wall opposite to the dark area facing its opening. The latency to enter the dark area (bottom left panel) is used as a parameter for initial activity in the light–dark box. For this parameter, there was no difference between the two strains, whereas flesinoxan reduced initial activity. The relatively large variance is related to the fact that some animals move into the dark area immediately, whereas others hardly move throughout the 10-min test (see also Bouwknecht and Paylor, 2002).

In the open-field test, vertical activity or rearing was scored automatically every time the elevated beams were broken. For the light–dark exploration test, rearing was scored manually (Fig. 2, bottom right panel). The effects of flesinoxan and strain were similar in both paradigms. The two strains responded differently to flesinoxan. B6 mice reared far more than S6 mice and the reduction in rearing after flesinoxan treatment was only significant in B6 mice. The lack of a significant effect in S6 mice was again probably due to a floor effect.

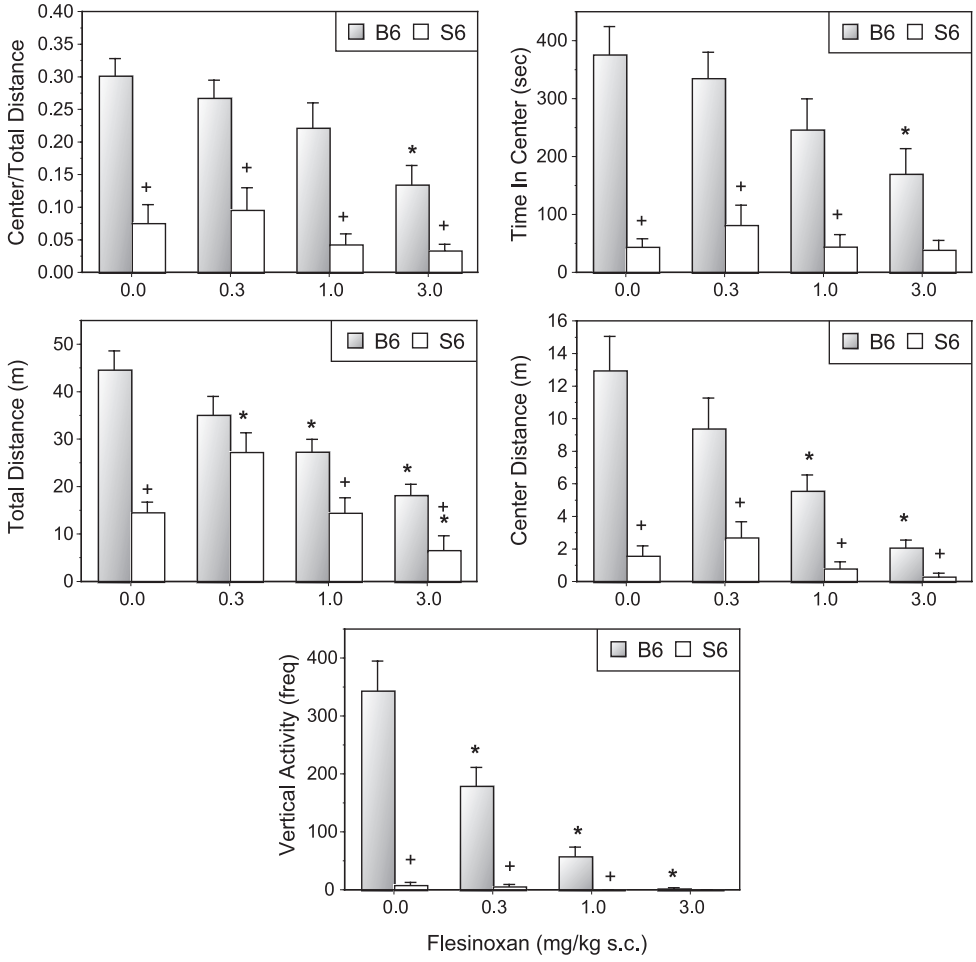

Fig. 1. Open-field activity test in C57BL/6J (B6) and 129S6/SvEvTac (S6) mice. Mice ($n = 10$ per dose) were tested using a balanced within-subject design in which each animal received each dose once with 1-week intervals. Bars represent mean ± S.E.M. for each respective parameter measured; symbol: *means significantly different ($P < 0.05$) from saline treatment, $^{+}$versus B6 mice. For detailed description of statistical analysis and overall effects, see Results. The parameters center/total distance and time in center are anxiety-related measures; the total distance, distance in center and vertical activity are parameters reflecting locomotion and exploration.

3.3. Stress-induced hyperthermia paradigm

In the stress-induced hyperthermia paradigm, the measure for anxiety is the increase in temperature (ΔT) over a 10-min window in response to the mild stress of measuring rectal temperature (Fig. 3). Flesinoxan reduced basal temperature (T_1) in both strains, whereas B6 mice are more sensitive than S6 mice. The stress-induced hyperthermia was reduced in both strains by flesinoxan, whereas this anxiolytic-like effect reached significance at a lower dose in S6 mice compared to B6 mice.

4. Discussion

In the current set of experiments, we focused on the influence of genetic background in mice on the anxiolytic effect of acute 5-HT_{1A}-receptor activation in three anxiety paradigms. In general, we hypothesized that an animal with a low anxiety level is expected to benefit less from treatment with a putative anxiolytic drug like flesinoxan than an anxious mouse. In a study on eight different substrains of 129 mice versus B6 mice, anxiety levels measured in the zero-maze were the highest in the 129 substrain that we selected for the present study, i.e. the S6 mice (Cook et al., 2002). Thus, we hypothesized that the putative anxiolytic effects of the 5-HT_{1A} receptor agonist flesinoxan would be seen across all paradigms in the high-anxiety strain (129S6/SvEvTac (S6), with limited or no effect in the low-anxiety strain (C57BL/6J (B6).

We used a multi-paradigm approach to assess anxiety in order to get a more complete picture of drug effects in relation to the innate differences in locomotor behavior between the two inbred strains. These paradigms are all based on unconditioned responses. We have recently shown in an extensive inbred mouse strain survey with two out of three assays used here that without drug treatment, mice can be used repeatedly at 1-week intervals (Bouwknecht and Paylor, 2002). A parallel study, which focused on consistency in subjects treated with the same

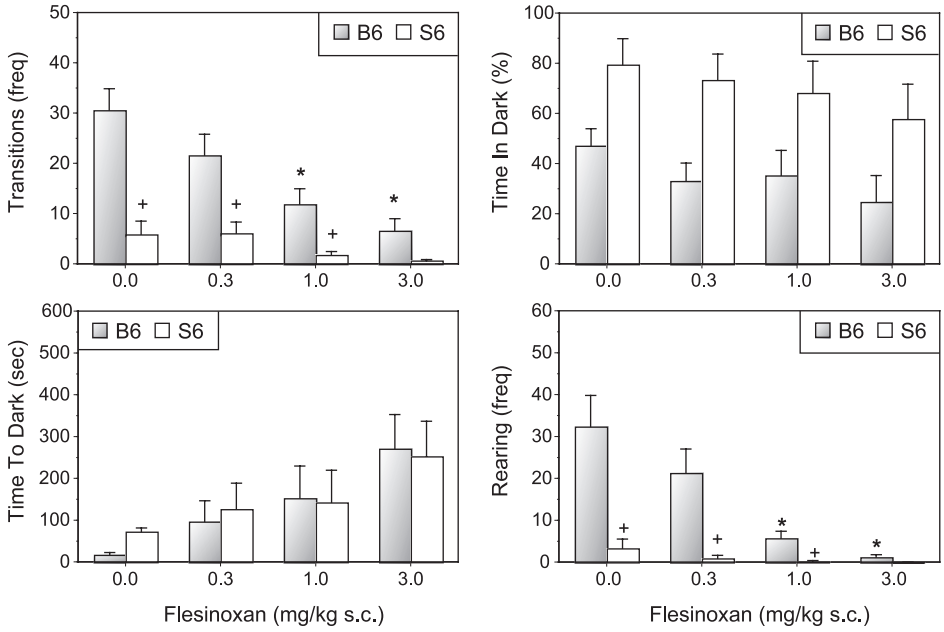

Fig. 2. Light–dark exploration test in C57BL/6J (B6) and 129S6/SvEvTac (S6) mice. Mice ($n = 10$ per dose) were tested using a balanced within-subject design in which each animal received each dose once with 1-week intervals. Bars represent mean ± S.E.M. for each respective parameter measured; symbol: *means significantly different ($P < 0.05$) from saline treatment, $^{+}$versus B6 mice. For detailed description of statistical analysis and overall effects, see Results. The parameters light–dark transitions and time in dark area are anxiety-related measures; time to dark is a measure for initial activity in the box and rearing is a measure for exploration.

dose of flesinoxan with 1-week intervals, showed that mice could be used repeatedly in drug studies (Bouwknecht et al., 2004). The present study describes a complete within-subject design for generating dose–response curves for flesinoxan.

The behavioral paradigms used in the present study, i.e. open-field activity and light–dark exploration test, are based on a natural aversion of mice for brightly lit environments (Crawley, 1985; Rodgers, 1997). Our findings in the open-field and light–dark test confirmed previous behavioral data

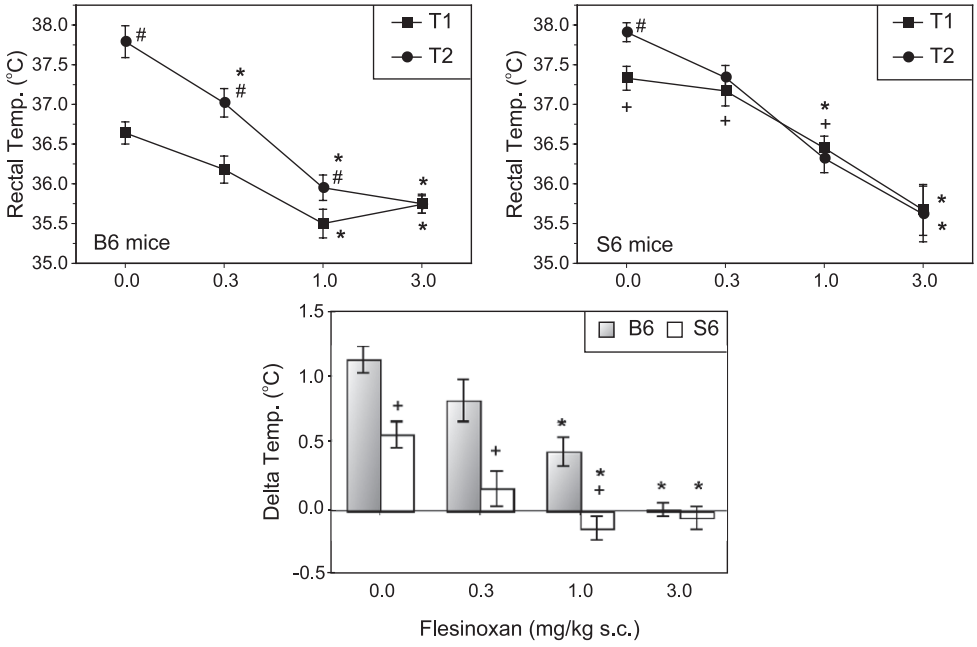

Fig. 3. Stress-induced hyperthermia paradigm in C57BL/6J (B6) and 129S6/SvEvTac (S6) mice. Mice ($n = 10$ per dose) were tested using a balanced within-subject design in which each animal received each dose once with 1-week intervals. Bars represent mean ± S.E.M.; symbols: *means significantly different ($P < 0.05$) from saline treatment, $^{+}$versus B6 mice and $^{\#}$versus T_1. For detailed description of statistical analysis and overall effects, see Results. The delta temperature is an anxiety-related measure, whereas T_1 reflects the drug effect on basal temperature.

in inbred strains showing that without drug-treatment B6 mice are less anxious and more active than S6 mice (Bouwknecht and Paylor, 2002; Contet et al., 2001; Cook et al., 2002; Homanics et al., 1999; Paulus et al., 1999; Rodgers et al., 2002; Voikar et al., 2001).

In general, flesinoxan induced dose-dependent behavioral responses in B6 mice in the behavioral paradigms, while effects were marginal in S6 mice possibly related to floor effects (Rodgers et al., 2002). The main behavioral measures for anxiety, center/total distance ratio, time in center area in the open field, and transitions in the light–dark test, unexpectedly suggest anxiogenic effects of flesinoxan in B6 mice. The center/total distance ratio in the open-field test is independent of locomotor activity. Flesinoxan reduced total distance and distance traversed in the center area to a different extent (two- versus five-fold), suggesting that 5-HT_{1A} receptor activation specifically induced anxiety in B6 mice. In contrast, the time spent in the dark area in the light–dark box is not affected by flesinoxan in either strain. An important difference between the light–dark and open-field test is the way mice can cope with the anxiogenic environment. The dark compartment in the light–dark box offers a safe environment, which is not available in the equally lit open-field box. Mice show thigmotaxis, i.e. wall hugging, as a safe alternative in the open field.

The initial reduced activity in the light–dark box, measured as latency to enter the dark compartment, together with the effects on locomotor activity suggests that flesinoxan may not only be anxiogenic in B6 mice but also sedative. At the highest dose, latency increased to almost 300 s, which is half the length of the light–dark assay. In contrast to the benzodiazepine-induced sedative low muscle tone and flat body posture, flesinoxan did not affect body posture (personal observation). Interestingly, flesinoxan completely abolished the vertical component of locomotor activity and exploration in B6 mice. In S6 mice, the lowest dose of flesinoxan caused hyperactivity, whereas the highest dose caused hypoactivity. In summary, data from the open-field and light–dark tests suggest that our original hypothesis was inaccurate because flesinoxan unexpectedly had no or limited effect in S6 mice and induced anxiety and hypoactivity in B6 mice.

In contrast to the behavioral responses, we obtained different results from the physiological stress-induced hyperthermia paradigm, which is thought to reflect a form of anticipatory anxiety (Borsini et al., 1989; van der Heyden et al., 1997). In the stress-induced hyperthermia paradigm, mice are tested in a familiar home cage instead of an anxious environment. The hyperthermic response was stronger in B6 than in S6 mice. Flesinoxan completely abolished the stress-induced hyperthermia response in both strains. In addition, B6 mice seemed more sensitive to the flesinoxan-induced hypothermia. These physiological data confirm previous findings in 129Sv mice showing anxiolytic effects of flesinoxan using radiotelemetric measurement of temperature and heart rate (Bouwknecht et al., 2000). The hyperthermia is stronger in an anxious light–dark box compared to a home cage, which suggests that body temperature has not reached a ceiling level in the standard home-cage setup and that familiarity with the test environment is an important factor (Bouwknecht and Paylor, 2002).

Overall, the current data in a within-subject design with each animal receiving each dose once showed nearly identical results compared with the between-subject design in which each animal was naive to the test procedure (Bouwknecht et al., 2004). Although direct statistical comparison is not possible, there are only a few apparent differences between the two designs. In the light–dark box, the frequency of transitions and the time to enter the dark in B6 mice are slightly lower in retested versus naïve mice. This suggests that pre-exposure to the box facilitates coping with the anxious environment by moving to the dark area and reducing the drive to explore the brightly lit area as supported by somewhat reduced rearing behavior. In the open-field test with B6 mice, the anxiety measures (center/total distance ratio and time in center) in the between-subject design showed significant anxiogenic effects at 1.0 versus 3.0 mg/kg flesinoxan in the within-subject design, which suggests that naive mice are slightly more sensitive to drug treatment. For all other parameters, the absolute levels in both strains and the effects of drug treatment were design-independent. Altogether, these effects of experimental design are very mild compared to the dramatic differences caused by genetic background and drug treatment.

At a mechanistic level, the role of 5-HT_{1A} receptors has been studied extensively in behavioral rodent models. In rats, it has been shown that flesinoxan reduces locomotion and in particular rearing in the open-field test, whereas the peripheral, horizontal activity increased (Ahlenius et al., 1991, 1993). This so-called serotonin syndrome is less obvious in mice. Low doses of 5-HT_{1A} receptor agonists might be anxiolytic, whereas high doses are anxiogenic and/or sedative (Borsini et al., 1999; Griebel et al., 1992; Misslin et al., 1990). We found such ‘‘bell-shaped dose–response curves’’ for total distance and time in center area in the open-field in the anxious S6 mice, whereas B6 mice showed increased anxiety and reduced locomotion.

Part of the complexity with the 5-HT_{1A} receptor system is its localization both as pre- and post-synaptic receptors, with opposite effects on 5-HT neurotransmission (Hillegaart et al., 1996; Pineyro and Blier, 1999). Increased 5-HT transmission leads to anxiogenic responses, whereas decreased 5-HT levels cause anxiolytic effects (Artaiz et al., 1998). However, local activation of postsynaptic 5-HT_{1A} receptors induced anxiolytic responses in rats (Kostowski et al., 1989; Stefanski et al., 1993). Some of these inconsistent findings may be species dependent, although both anxiolytic and anxiogenic effects have been found in rats and mice after 5-HT_{1A} receptor activation (for review, see the work of Olivier and Miczek, 1999).

The development of mutant mice lacking 5-HT_{1A} receptors has given us new and important tools to study such

complicated mechanisms (for review, see Gingrich and Hen, 2001). Currently, there are three 5-HT_{1A} receptor knockout mice lines available generated on different backgrounds (C57BL/6 Heisler et al. (1998), 129Sv Ramboz et al. (1998), and Swiss-Webster Parks et al. (1998)). Interestingly, not only the low-anxiety strains (C57BL/6J and Swiss-Webster (van Gaalen and Steckler, 2000), but also the high-anxiety 129Sv strain (Homanics et al., 1999) displayed anxiogenic phenotypes, although there seems to be little margin for an increased anxiety response in the latter strain. So, all three strains of 5-HT_{1A} receptor knockout mice showed anxiogenic responses in one or more behavioral and/or physiological assays (Groenink et al., 2003; Olivier et al., 2001). A recent study on inducible and tissue-specific 5-HT_{1A} receptor knockout mice on a mixed background (Gross et al., 2002) found that rescue of postsynaptic 5-HT_{1A} receptors in the forebrain reverses the anxiogenic effect seen in the open field in the knockout mice. Absence of frontal 5-HT_{1A} receptors during the early postnatal period caused an irreversible anxiogenic state in these mice (Gross et al., 2002), which elucidates the important role of 5-HT_{1A} receptors in development of anxiety and stress responses. Altogether, these findings show the complex influence of factors such as genetic background, development, and localization of 5-HT_{1A} receptors.

One of our reasons to compare high- and low-anxiety mice is related to the fact that studying clinical anxiety in humans is different from drug studies in healthy animals (see review of Haller, 2001). It is important to select the appropriate mouse strain and paradigm as a model for human diseases (Crawley et al., 1997). Drug responses are also strain dependent (Crawley et al., 1997), whereas the effects of anxiolytic compounds can be unrelated to initial anxiety levels in inbred strains (Belzung and Griebel, 2001) and behavior in one anxiety paradigm does not always predict anxiety levels in other paradigms (Cook et al., 2002). It should be noted here that acute treatment with a 5-HT_{1A} compound might induce different effects and involve other systems than after chronic treatment or gene manipulation. Further research on the influence of genetic background as a predictor for anxiolytic or anxiogenic responses in inbred mice is necessary. It is likely that this concern will generalize to drugs that affect other behavioral phenotypes.

Altogether, this study revealed important differences in behavioral versus physiological responses to 5-HT_{1A} receptor activation in anxiety-related paradigms in the two mouse strains. The hypothesis that anxiolytic-like effects of the 5-HT_{1A} receptor agonist flesinoxan would be evident in a high-anxiety strain (S6) and limited or absent in a low-anxiety strain (B6) appeared inaccurate. In summary, acute treatment with flesinoxan appeared to have anxiogenic-like effects in the behavioral paradigms and anxiolytic-like effects in the physiological assay. Flesinoxan also reduced locomotion in B6 mice, suggesting a sedative effect. Additional studies using chronic instead of acute treatment with flesinoxan may elucidate some of the apparent unexpected and contradicting results found in this study. Our data further emphasize the importance of genetic make up and favor a multi-paradigm approach using both behavioral and physiological assays in order to properly characterize phenotypic effects in mutant mice.

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