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ORIGINAL INVESTIGATION

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Individual differences in response to imipramine in the mouse tail suspension test

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Abstract The tail suspension test is a behavioural primary screen for detecting potential antidepressant drugs. In this test, a reduction of duration of immobility after treatment with imipramine is obtained in mice of the NMRI strain but not of the CD1 strain. The present experiments evidence important differences between individuals of the latter strain in both the amount of immobility observed in naive mice and the effects of three antidepressants. The reproducibility of the tail suspension-induced behavioural despair was high in individual CD1 male mice and allowed a preselection of spontaneous high and low immobility scorers. Only the high immobility scorers were responsive to imipramine (30 mg/kg), desipramine (30 mg/kg) and paroxetine (10 mg/kg). The percentage of spontaneous high immobility scorers was higher in NMRI (50%) than in CD1 (20%) mice, justifying the use of the former strain for screening potential antidepressants. However, controlling for individual differences in the spontaneous performance in this animal model of depression may provide a useful tool to study behavioural, neurochemical and neuroendocrine correlates of antidepressant action.

Key words Tail suspension test · Preselection · Mouse strains · Imipramine · Desipramine · Paroxetine

Introduction

Screening tests for antidepressants are either behavioural or based on drug interactions (Willner 1990). In behavioural tests, the animals are generally placed in aversive situations which induce recognizable behavioural changes such as immobility. The effects of drugs on the induced behavioural changes are evaluated. Rodents (rats or mice) when forced to swim in a restricted space from which they cannot escape will, after an initial period of activity, adopt a characteristic immobile posture called "behavioural despair" (Porsolt et al. 1977, 1978, 1993). The tail suspension test has been more recently proposed where immobility is induced in mice simply by suspending them, for short periods, by the tail (Stéru et al. 1985). This test has been automated (ITEMATIC-TST) and measures duration of immobility and the power of the movements of mice (Porsolt et al. 1993). The tail suspension procedure bypass several problems of the swimming model: the immobility is objectively measured; no hypothermia is induced by immersion in cold water, and thus it is considerably less stressful to experimental animals than the forced swimming test (Thierry et al. 1986).

Marked differences exist between strains in both the amount of immobility observed and the effects of a standard antidepressant drug imipramine. For example, baseline duration of immobility is higher in male CD1 than in NMRI mice in the forced swim test (Porsolt et al. 1978). The reverse situation occurs in the tail suspension test (van der Heyden et al. 1987). Moreover, no effects of imipramine could be observed by van der Heyden et al. (1987) when using CD1 mice.

Marked individual differences also exist within strains. Our recent findings based on a selective breeding strategy of spontaneous "helpless" or "non-helpless" CD1 mice show that performance in the mouse tail suspension test is under specific genetic control. Our results also demonstrate that animals prone to be helpless but not "healthy" controls are sensitive to an antidepressant (Vaugeois et al. 1996).

The purpose of this study was to find out whether the previously reported lack of efficacy of imipramine in the tail suspension test might be due to the low degree of immobility of control mice, and to examine if the antidepressive-like effect of imipramine, a mixed serotonin and noradrenaline reuptake inhibitor, could be demonstrated in mice classified as "high-immobility" animals after a preselection. We also investigated whether individual differences in performance during this procedure were

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stable. Finally, the noradrenaline reuptake inhibitor desipramine and the selective serotonin reuptake inhibitor paroxetine were tested in order to confirm the usefulness of this selection procedure.

Materials and methods

Animals

This study was in accordance with the Guidelines for the Use of Animals in Research (French Decree n° 87.848). Two strains of male mice were used: Swiss albino CD1 mice (Charles River, Saint-Aubin lès Elbeuf, France) and NMRI mice (Iffa Credo, L'arbresle, France) weighing 17 ± 1 g when purchased. They were housed in groups of 20 mice in Makrolon cages with free access to food and water. Testing was performed between 0900 hours and 1700 hours during the light (0700–1900 hours) part of the day-night cycle.

Drugs

Imipramine (Ciba Geigy, France) was used in one experiment at the 30 mg/kg dose, which was determined as effective in NMRI mice in a previous study (van der Heyden et al. 1987). Imipramine or NaCl 0.9% was injected IP 30 min prior to testing in a volume of 0.2 ml per 20 g body weight. Paroxetine (Smithkline Beecham, France) and desipramine (Ciba Geigy, France) were dissolved in water and used at 10 mg/kg and 30 mg/kg, respectively.

Tail suspension test

A computerized device (ITEMATIC-TST) developed by ITEM-LABO (Le Kremlin-Bicêtre, France) was used to measure the total sum of periods of immobility (duration of immobility) in the tail suspension test. Mice were suspended by the tail, using adhesive Scotch tape, to a hook connected to a strain gauge that picked up all movements of the mouse and transmitted them to a central unit which calculated the total duration of immobility during a 6-min test. Six animals were tested at one time.

Forced swim test

The apparatus consisted of two Plexiglas cylinders (25 cm height, 10 cm internal diameter) placed side by side in a Makrolon cage (38 × 24 × 18 cm) filled with water (8 cm height) at 21–23°C. Two mice were tested simultaneously for a 6-min period, but a non-transparent screen placed between the two cylinders prevented mice from seeing each other. A mouse was judged to be immobile when it remained floating in the water, making only those movements necessary to keep its head above water.

Statistics

Statistical analysis of the results was performed using ANOVA, χ^2 test or Student's *t*-test.

Results

In the tail suspension test performed with CD1 mice randomly removed from a cage, a pilot study showed no statistically significant decrease in duration of immobility after treatment with imipramine (30 mg/kg): 35 ± 9 as compared to saline: 59 ± 6 (mean ± SEM of groups of nine mice, $P > 0.05$). The durations of immobility in the 6-min observation period in the tail suspension test in 60 naive male CD1 or NMRI mice are shown in Fig. 1. The immobility times were 79 ± 6, ranging from 6 to 199 s, for CD1 mice and 111 ± 7 (Student's *t*-test, $P < 0.001$), ranging from 9 to 218 s, for NMRI mice. The distributions were unimodal, although skewed to the left with CD1 mice, and differed significantly between each other (χ^2 test, $P < 0.01$). Hence, 20% of CD1 mice had a score below 35 s as compared to 8% of NMRI mice. The percentage of CD1 mice that had a score equal or greater than 115 s was higher in NMRI (50%) than in CD1 (20%) mice. By comparison, the duration of immobility in the 6-min observation period in the forced swim test performed later on the same 60 male CD1 or NMRI mice are also shown in Fig. 1. The immobility times were 226 ± 6, ranging from 100 to 301 s, for CD1 mice and 207 ± 7 (Student's *t*-test, $P < 0.05$), ranging from 21 to 286 s for NMRI mice. The distributions did not differ

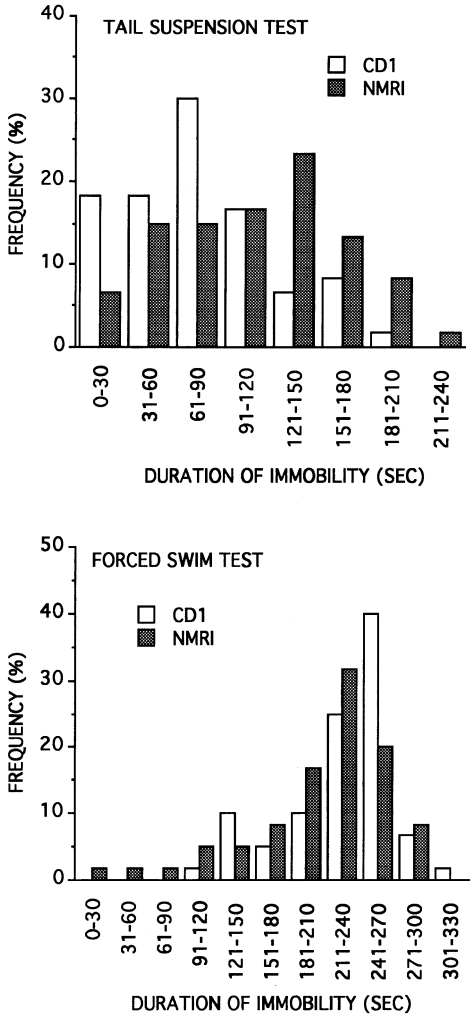

Fig. 1 Duration of immobility recorded in the tail suspension test and in the forced swim test. The forced swim test took place 4 weeks after the tail suspension test. Data are expressed as the frequency distribution of 60 naive male CD1 or NMRI mice. Immobility was recorded for a 6-min period in both tests

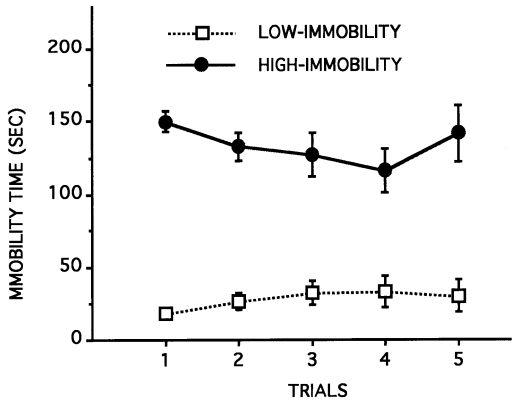

Fig. 2 Immobility times of male CD1 mice tested daily during 5 consecutive days. On first trial, scores of "low-immobility" mice (*open squares*) were less or equal to 15 s and scores of "high-immobility" mice (*closed circles*) were above or equal to 115 s. Data are mean ± SEM values obtained from 12 mice per group

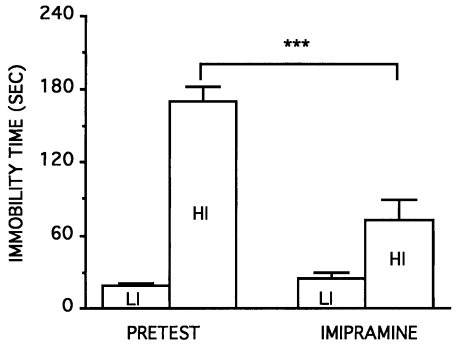

Fig. 3 The influence of pretest selection on imipramine effects in the tail suspension test in male CD1 mice. Pretest session consisted of one trial on 2 consecutive days. For each selected mouse: "low-immobility" mouse (*LI*) or "high-immobility" mouse (*HI*), the mean score was calculated and used as the pretest score. On day 3, mice were injected with imipramine (30 mg/kg IP) 30 min before the test. Data are mean ± SEM values obtained from eight mice per group. *** Denotes statistical significant difference ($P < 0.001$) from pretest value

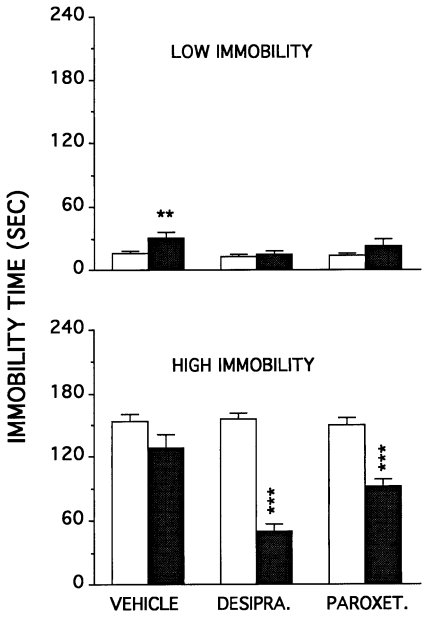

Fig.4 The influence of pretest selection on desipramine or paroxetine effects in the tail suspension test in male CD1 mice. Pretest selection was the same as in Fig. 3. On day 3, mice were injected with desipramine (30 mg/kg IP), paroxetine (10 mg/kg IP) or vehicle (water IP) 30 min before the test. The *open bars* show the duration of immobility in the pretest and the *dotted bars*, that in the test. Data are mean ± SEM values obtained from 17 (vehicle) or 15 (treatments) mice. *Closed asterisks* denote a significant effect of vehicle (** $P < 0.01$) or drug (*** $P < 0.001$) administration

significantly between each other (χ^2 test, $P = 0.07$). However, 10% of NMRI mice had a score below 115 s as compared to only 1.7% of CD1 mice.

From the above tail suspension test carried out in CD1 mice, we decided arbitrarily that the 20% of mice (i.e. 12 mice) showing the lowest immobility times would be considered as "low-immobility" (LI, score ≤35 s) and the 20% showing the highest immobility times (i.e. 12 mice) would be considered as "high-immobility" animals (HI, score ≥115 s). These HI or LI mice were tested again for 4 more consecutive days. A one-way ANOVA revealed no significant time effect ($P > 0.05$) across trials in either HI or LI groups of mice (Fig. 2).

In another experiment, 46 LI and 37 HI mice were selected on day 1 from a sample of 186 tested mice. On the following day, from 46 LI tested mice 20 mice scored below 35 s, whereas from 37 HI mice 25 mice scored over 115 s. On day 3, mice were injected 30 min before the test with either NaCl 0.9% or imipramine (30 mg/kg). When the results of HI and LI mice were considered separately, the tricyclic antidepressant was seen to induce a decrease in the immobility score in the HI group and was ineffective in the LI group (Fig. 3). On day 3, scores of vehicle-injected mice did not change in comparison with mean scores in trials 1 and 2 both in LI mice: 21 ± 3 versus 24 ± 2, respectively (mean ± SEM of groups of eight mice, $P > 0.05$), and in HI mice: 159 ± 7 versus 150 ± 16, respectively (mean ± SEM of groups of 12 mice, $P > 0.05$). Observations throughout this experiment indicated that the percentage of mice that defecated during the test was twice in the HI group in comparison to the LI group (21.6% versus 10.9% on day 1).

Desipramine (30 mg/kg) or paroxetine (10 mg/kg) were also administered in mice preselected according to the procedure used to study the effects of imipramine. Both drugs decreased the duration of immobility in HI mice, but had no significant effects upon the behaviour of LI mice in the tail suspension test. Whereas the injection of vehicle did not alter the immobility in the HI group, a significant increase in the immobility time was observed in the LI group used in this last experiment (Fig. 4).

Discussion

The tail suspension test in mice was originally described by Stéru et al. (1985) as a screening procedure

for evaluating antidepressant activity of drugs inspired by Porsolt's "behavioural despair" test or forced swim test. Both tests are based on the principle that mice, exposed to an aversive situation from which there is no escape, will, after periods of agitation, cease attempts to escape and become immobile. Many clinically effective antidepressants reduce the immobility that mice display in both procedures. One important class of antidepressants showing clear activity in the tail suspension test but little activity in the forced swim test is that of the selective serotonin reuptake inhibitors, which differentiate the tail suspension test from "behavioural despair" test (Porsolt et al. 1993). Another difference between the two tests was suggested by the fact that imipramine clearly decreases the duration of immobility displayed by NMRI mice in the tail suspension test but shows no activity when using CD1 mice (van der Heyden et al. 1987). Reciprocally, however, imipramine reduces the duration of immobility displayed by CD1 mice in the forced swim test but shows no activity when using NMRI mice. The present experiments suggest that individual differences in behaviour could explain this distinct sensitivity to imipramine between the two strains in these tests.

The results obtained here are fully consistent with those previously reported by van der Heyden et al. (1987). First, we confirm the lack of efficacy of imipramine in the tail suspension test when using CD1 mice. Second, the baseline duration of immobility is higher in male NMRI than in CD1 mice in the tail suspension test as previously demonstrated by these authors. We observed in two experiments that repeated testing permitted a preselection of spontaneous high immobility scorers called "high-immobility" mice. Only these "high-immobility" mice would respond to the administration of imipramine. Two other antidepressants with differing mechanisms of action, the noradrenaline reuptake inhibitor desipramine and the selective serotonin reuptake inhibitor paroxetine, decreased the duration of immobility only in "high-immobility" mice. Previous studies using unselected NMRI mice had already mentioned the activity of desipramine (Stéru et al. 1985) and paroxetine (Perrault et al. 1992) in the tail suspension test. This strongly suggests that the low baseline duration of immobility observed with the majority of individuals in the CD1 strain precludes the observation of the expected effect after treatment by imipramine and other antidepressants.

The reverse situation occurs in the forced swim test, i.e. the baseline duration of immobility is higher in male CD1 than in NMRI mice (Porsolt et al. 1978). Our data are also fully consistent with those previously reported by Porsolt and collaborators, and suggest that the same explanation would satisfactorily fit with the observed results.

We observed that "high-immobility" mice defecated more than "low-immobility" mice. For rats, defecation scores in an open field is considered to be a good measure of emotionality in aversive situations (Soubrié et al. 1974). In line with our findings, Maudsley reactive rats, which have been genetically selected for their high defecation rate in an open field and which are considered highly emotional, showed a higher degree of immobility in the forced swimming test than their counterparts, the Maudsley non-reactive rats (Abel 1991). However, further experiments would be performed in order to search for a relationship between emotionality and immobility in the tail suspension test, because the increased defecation rates exhibited by rats in various tests were not indicative of a general increase in emotionality in other situations (Armario et al. 1988; Abel 1991).

In one experiment, a vehicle injection altered the behaviour of "low-immobility" mice, indicating that injections might differently affect mice responding to mild stressors with opposite active or passive strategies. The generalization of this finding and the mechanisms underlying this effect remain to be determined.

The strain factor should be taken into account in attempts to replicate results from one laboratory to another. Mice of the CD1 strain exhibited marked variations in their response in the tail suspension test. This may explain the lack of efficacy of the antidepressant imipramine previously reported in another study (van der Heyden et al. 1987).

A preselection procedure of animals makes the tail suspension test feasible with CD1 mice. Many mice showed a remarkable stability in their response when retested. Stable behaviours have also been previously observed in the elevated plus maze test (Lister 1987) but not others. In fact, it has been shown that rodents' immobility in the forced swimming test may increase with experience of the test (Nagatani et al. 1984; Armario et al. 1988; De Pablo et al. 1989; Marti and Armario 1993), suggesting the existence of a learned helplessness phenomenon. However, the present results could be explained by assuming that the helplessness of mice in the tail suspension test is a trait rather than a state-dependent marker.

Antidepressant drugs alleviate depressive symptomatology in depressed patients but not in healthy persons. For animal experimentation aimed at screening antidepressants, this has the following consequences: genetically and/or environmentally manipulated models would be closer to the clinical situation than models based on standard laboratory strains. The present results showed that imipramine was active after a preselection procedure which retained spontaneous "high-immobility" CD1 mice. Interestingly, evidence from family and twin studies suggests that genetic factors play a role in the development of affective disorders (Kendler et al. 1994). The selectively bred lines of spontaneous "helpless" and "non-helpless" CD1 mice (Vaugeois et al. 1996) may also provide a novel approach to investigate behavioural, neurochemical and neuroendocrine correlates of antidepressant action.

References

Abel EL (1991) Behavior and corticosteroid response of Maudsley reactive and nonreactive rats in the open field and forced swimming test. Physiol Behav 50: 151–153

Armario A, Gavalda A, Marti O (1988) Forced swimming test in rats: effect of desipramine administration and the period of exposure to the test on struggling behavior, swimming, immobility and defecation rate. Eur J Pharmacol 158: 207–212

De Pablo JM, Parra A, Segovia S, Guillamon A (1989) Learned immobility explains the behavior of rats in the forced swimming test. Physiol Behav 46: 229–237

Kendler KS, Walters EE, Truett KR, Heath AC, Neale MC, Martin NG, Eaves LJ (1994) Sources of individual differences in depressive symptoms: analysis of two samples of twins and their families. Am J Psychiatry 151: 1605–1614

Lister RG (1987) The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology 92: 180–185

Marti J, Armario A (1993) Effects of diazepam and desipramine in the forced swimming test: influence of previous experience with the situation. Eur J Pharmacol 236: 295–299

Nagatani T, Sugihara T, Kodaira R (1984) The effect of diazepam and of agents which change gabaergic functions in immobility in mice. Eur J Pharmacol 97: 271–275

Perrault G, Morel E, Zivkovic B, Sanger DJ (1992) Activity of litoxetine and other serotonin uptake inhibitors in the tail suspension test in mice. Pharmacol Biochem Behav 42: 45–47

Porsolt RD, Bertin A, Jalfre M (1977) Behavioural despair in mice: a primary screening test for antidepressants. Arch Int Pharmacodyn Ther 229: 327–336

Porsolt RD, Bertin A, Jalfre M (1978) "Behavioural despair" in rats and mice: strain differences and the effect of imipramine. Eur J Pharmacol 51: 291–294

Porsolt RD, Mc Arthur RA, Lenègre A (1993) Psychotropic screening procedures. In: Van Haaren F (ed) Methods in behavioral pharmacology. Elsevier, Amsterdam, pp 23–51

Soubrié P, Wlodaver C, Schoonhoed L, Simon P, Boissier JR (1974) Preselection of animals in studies of anti-anxiety drugs. Neuropharmacology 13: 719–728

Stéru L, Chermat R, Thierry B, Simon P (1985) The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology 85: 367–370

Thierry B, Stéru L, Simon P, Porsolt RD (1986) The tail suspension test: ethical considerations. Psychopharmacology 90: 284–285

Van der Heyden JAM, Molewijk E, Olivier B (1987) Strain differences in response to drugs in the tail suspension test for antidepressant activity. Psychopharmacology 92: 127–130

Vaugeois J-M, Odièvre C, Loisel L, Costentin J (1996) A genetic mouse model of helplessness sensitive to imipramine. Eur J Pharmacol 316: R1–R2

Willner P (1990) Animal models of depression: an overview. Pharmacol Ther 45: 425–455