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Effects of the antidepressants desipramine and fluvoxamine on latency to immobility and duration of immobility in the forced swim test in adult male C57BL/6J mice

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Abstract

The forced swim test in rodents allows rapid detection of substances with antidepressant-like activity, evidenced as a decreased duration of immobility that is produced by the majority of clinically used antidepressants. Antidepressants also increase the latency to immobility, and this additional measure reportedly can increase the sensitivity of the forced swim test in mice. Extending these findings, the present study examined the effects of desipramine and fluvoxamine in a forced swim test in C57BL/6J mice, a strain commonly used as background for genetic modifications, analyzing results with a method (i.e., survival analysis) that can model the skewed distribution of latencies and that can deal with censored data (i.e., when immobility does not occur during the test), in comparison with the more traditional Student's t-test. Desipramine increased the latency to immobility at 32 mg/kg, but not at lower doses. Fluvoxamine also did not affect latency at lower doses, but in contrast to desipramine, fluvoxamine decreased the latency to immobility at the highest dose (i.e., 32 mg/kg). At doses affecting latency to immobility, neither desipramine nor fluvoxamine significantly affected duration of immobility. Together, these results are generally consistent with the suggestion that inclusion of the latency measure can increase the sensitivity of the forced swim test to detect antidepressant-like effects in mice.

Keywords

desipramine; fluvoxamine; antidepressant; forced swim test; immobility; latency; duration; C57BL/6J mouse; Kaplan-Meier survival analysis

Introduction

The forced swim test in rodents allows rapid detection of substances with antidepressant-like activity, evidenced as a decreased duration of immobility that is produced by the majority of clinically used antidepressants (e.g., Petit-Demouliere et al., 2005; Castagné et al., 2010). Antidepressants also increase the latency to immobility, and this additional measure can increase the sensitivity of the forced swim test in mice, evidenced by drug effects on latency occurring at lower doses than drug effects on duration (Castagné et al., 2009). Extending these findings, the present study examined the effects of the tricyclic antidepressant desipramine and the selective serotonin reuptake inhibitor (SSRI) fluvoxamine on latency to immobility, and on duration of immobility, in a forced swim test in C57BL/6J mice, a strain commonly used for genetic modifications. Fluvoxamine was selected as a negative control, because in contrast with tricyclic antidepressants, SSRIs generally do not affect immobility in the forced swim test in C57BL/6J mice (e.g., Lucki et al., 2001; Castagné et al., 2009).

Distributions of latencies are often skewed to the right (i.e., with many values toward the left and only a few toward the right, resulting in the mean being greater than the median) instead of being symmetrical with the mean equal to the median. Therefore, latency to immobility data were analyzed using survival analysis, which can model the skewed distribution of latencies and deal with censored data (i.e., when immobility does not occur during the test). To facilitate comparison with previously reported results, latency to immobility data, and duration of immobility data, were analyzed also by Student's t-test as described by Castagné et al. (2009). Survival analysis results were compared with those obtained by the more traditional t-test to examine the robustness of the t-test, which assumes symmetry and normality, to deviations of normality and to censoring of latency data in the forced swim test.

Methods

Subjects

Adult male C57BL/6J mice, originally acquired from The Jackson Laboratory (Bar Harbor, ME) were bred in house and maintained in a temperature-controlled (24 °C) vivarium on a 12/12-hour light/dark cycle (lights on at 07:00 h) in groups of five in plastic cages (29 × 18 × 13 cm) with bedding and with free access to food and water. Behavioral testing was conducted in accordance with guidelines of the National Institutes of Health (NIH) and the local Institutional Animal Care and Use Committee; at the time of testing, the median age was 20 weeks (range: 11–28).

Procedure

The forced swim test was conducted using a procedure similar to that detailed elsewhere (Castagne et al., 2011). Briefly, mice were individually placed for 6 min in a cylinder (height=25 cm; diameter =20 cm) containing 15 cm water (24 °C) from which they could not escape. Test sessions were videotaped using a camera positioned directly above the cylinder. Latency to immobility [defined as cessation of all active behaviors and remaining passively floating, or making minimal movements necessary to maintain the nostrils above

water, for at least one second (Castagné et al., 2009)], and duration of immobility during the last 4 min of the 6 min test, were measured in s by an observer unaware of the treatment conditions. Mice were given an injection of saline or 3.2, 10, or 32 mg/kg desipramine or fluvoxamine, 30 min before the test. Doses of desipramine and fluvoxamine were selected on the basis of results in male C57BL/6J mice reported by Lucki et al. (2001) and Horton et al. (2013), respectively. In anticipation of future drug interaction studies, all mice also received an injection of saline 60 min before the test.

Desipramine, but not fluvoxamine, has been examined previously in the forced swim test in C57BL/6J mice (Lucki et al., 2001). Estimated from the results shown in Fig. 1 of Lucki et al. (2001), desipramine decreased mean immobility by 45 s, which, divided by a standard deviation of 30, yields an effect size of 1.5. To detect such an effect with 80% power requires 9 animals per group [power analysis for Student t-test; G*Power software, Faul et al., 2007]. Thus, a sample size of 9 was considered adequate to replicate and extend previously reported effects of desipramine on duration of immobility.

Drugs

Desipramine (desmethylinipramine) hydrochloride and fluvoxamine maleate (Sigma-Aldrich, St. Louis, MO) were dissolved in physiological saline and injected i.p. at doses expressed as base weight per kg body weight. The injection volume was 10 ml/kg.

Data analysis

Because of non-normal distribution of latencies to immobility, and the occurrence of censoring (i.e., animals that did not show immobility during the entire 6-min test period), data were analyzed by Kaplan-Meier survival analysis (e.g., Powell et al., 2012), conducted separately for each drug. Differences between groups were examined by the Mantel-Cox log-rank test and by the Gehan-Breslow-Wilcoxon test, which gives more weight to early time points. To facilitate comparison with previously reported results, latency to immobility and duration of immobility during the last 4 min of the test were analyzed also by Student's t-test as described by Castagné et al. (2009). Differences were considered statistically significant when $p < 0.05$. All analyses were performed using Prism 7.03 (GraphPad, San Diego, CA).

Results

Latencies to immobility had a skewed distribution (data not shown; median: 117 s; mean: 123 s; skewness: 1.3; kurtosis: 2.4) that deviated significantly ($p=0.0036$) from normality (D'Agostino and Pearson test). Censoring (i.e., not showing immobility during the entire 6-min test period) occurred in 6% of the mice tested. Due to this non-normal distribution, and due to censoring, latency data were analyzed by Kaplan-Meier survival analysis. Saline control data obtained in experiments with desipramine and fluvoxamine did not differ significantly (Mantel-Cox log-rank test: $p=0.11$; Gehan-Breslow-Wilcoxon test: $p=0.20$) and were combined in subsequent analyses. The time it took for 50% of the saline controls to show immobility was 117 s (Fig. 1, panels A and D). The latency to immobility was significantly increased by the highest dose of desipramine (Fig. 1, panel A; Table 1), and

was significantly decreased by the highest dose of fluvoxamine (Fig. 1, panel D; Table 1). Lower doses of either drug did not have statistically significant effects on latency to immobility. Mean latencies analyzed by Student's t-test were significantly increased by the highest dose of desipramine (Fig. 1, Panel B; Table 1) and were not significantly changed by fluvoxamine (Fig. 1, panel E; Table 1). None of the differences in duration of immobility between drug-treated groups and saline control (Fig. 1, panels C and F) reached statistical significance, other than the difference between 3.2 mg/kg desipramine and saline (Table 1).

Discussion

The present finding that desipramine increased the latency to immobility in the forced swim test extends previous observations by Castagné et al. (2009) who examined effects of imipramine in male C57BL/6J mice. This increased latency was observed at a dose of desipramine (32 mg/kg) that did not affect total immobility, consistent with the suggestion by Castagné et al. (2009) that the latency measure increases the sensitivity of the mouse forced swim test to detect antidepressant-like activity. However, inconsistent with this suggestion, 3.2 mg/kg desipramine decreased duration of immobility without affecting latency to immobility. It is surprising that desipramine decreased immobility only at 3.2 mg/kg, because under generally similar conditions [e.g., same strain (C57BL/6J), same sex (male), same route of administration (ip), same injection – test interval (30 min), same test duration (6 min)] desipramine has been found to decrease immobility at 5, 10, and 20 mg/kg (Lucki et al., 2001). However, the animals used in the present study were older than those used by Lucki et al. (2009) (median age 20 and 11 weeks, respectively). Previously, age-dependent changes in sensitivity of mice to desipramine within the adult period have been observed in the tail suspension test of antidepressant-like activity, with desipramine being less potent in older-than in younger adults (43 and 13 weeks, respectively) (Mitchell et al., 2015). Future studies of the possible age-dependency of the effects of desipramine in the mouse forced swim test could perhaps help to explain the discrepancy between the desipramine results obtained here and by Lucki et al. (2001). Be that as it may, it would also be of interest to examine if variability among the outcomes of different studies could be reduced by measuring latency to immobility in addition to its duration.

Based on the distribution of the durations of all bouts of immobility during the forced swim test, which differed among mice, Chen et al. (2015) determined a threshold for each animal, and calculated the latency to immobility as the latency to the first bout of immobility that was longer than the individual threshold. Taking such individual differences in immobility into account may help to reduce variability. Thus, future studies using analyses detailed by Chen et al. (2015) could help define thresholds for immobility (here 1 s, based on Castagné et al., 2009) that yield optimal sensitivity.

The observation that the SSRI fluvoxamine did not affect duration of immobility is consistent with previous findings using a different SSRI, fluoxetine, in C57BL/6J mice (Lucki et al., 2001; Castagné et al., 2009), and is further evidence that background strain is an important consideration in studies of antidepressant-like activity using the mouse forced swim test (Lucki et al., 2001). As expected, and consistent with its lack of effect on duration of immobility, fluvoxamine did not increase the latency to immobility. In contrast to

desipramine, the highest dose of fluvoxamine (i.e., 32 mg/kg) decreased the latency to immobility (in Fig. 1, compare panel D with A), perhaps related to abnormal swimming reportedly induced by a high dose of another SSRI, fluoxetine [i.e., 64 mg/kg (Castagné et al., 2009)]. Without measuring the latency to immobility and analyzing the latency data by survival analysis, effects of fluvoxamine would not have been detected in the present experiment.

Analyzing latency to immobility data with survival analysis and Student's t-test had similar outcomes for desipramine, but not for fluvoxamine. The different outcomes for fluvoxamine suggest that Student's t-test may not be robust to deviations of its underlying assumption of normality when used to analyze latency to immobility data. Future analysis of latency to immobility in the mouse forced swim test with both survival analyses and Student's t-test will help to further evaluate the robustness of Student's t-test to deviations of normality and to censoring of latency data.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Effects of desipramine (upper panels) and fluvoxamine (lower panels) on immobility of adult male C57BL/6J mice in the forced swim test. Panels A and D: Drug effects on latency to immobility, analyzed by Kaplan-Meier survival analysis. Data are expressed as the cumulative percentage of mice that started showing immobility during a 6 min session. Panels B and E: drug effects on latency to immobility, analyzed by Student's t-tests (Castagné et al., 2009). Panels C and F: drug effects on total immobility time during the last 4 min of the 6 min session, analyzed by Student's t-tests (Castagné et al., 2009). Desipramine: n=9–10 per group; fluvoxamine: n=7–8 per group; saline: n=26. Saline control data in panels D, E, and F are replotted from panels A, B, and C, respectively. * $p < 0.05$ (panels A and D: Gehan-Breslow-Wilcoxon test vs saline; panels B, C, E, F: t-test vs saline). Results of the statistical analyses are detailed in Table 1.

Results of statistical analyses examining effects of desipramine and fluvoxamine on latency to immobility and on duration of mobility time in the forced swim test in adult male C57BL/6J mice shown in Fig. 1. Latency to immobility was analyzed by 1) Kaplan-Meier survival analysis, and differences between groups were examined by the Mantel-Cox long-rank test and by the Gehan-Breslow-Wilcoxon test, which gives more weight to early time points. Latency to immobility was analyzed also by Student's t-test as described by Castagné et al. (2009), as was duration of immobility during the last 4 min of the 6 min test.

Table 1

	latency to immobility					duration of immobility				
	Mantel-Cox log-rank					Gehan-Breslow-Wilcoxon				
	χ^2	df	p			χ^2	df	p	t-test	p
desipramine (mg/kg) vs saline										
3.2	1.81	1	0.18			2.74	1	0.098	0.11	0.029
10	2.00	1	0.16			3.11	1	0.078	0.11	0.42
32	2.85	1	0.092			5.89	1	0.015	0.04	0.59
fluvoxamine (mg/kg) vs saline										
3.2	1.95	1	0.16			0.03	1	0.86	0.21	0.11
10	0.57	1	0.45			0.06	1	0.81	0.37	0.30
32	4.67	1	0.031			4.21	1	0.040	0.15	0.49