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Biphasic effects of selective serotonin reuptake inhibitors on anxiety: rapid reversal of escitalopram's anxiogenic effects in the novelty-induced hypophagia (NIH) test in mice?

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Abstract

In humans, chronic treatment with selective serotonin reuptake inhibitors (SSRIs) decreases anxiety, unlike acute treatment, which can increase anxiety. Although this biphasic pattern is observed clinically, preclinical demonstrations are rare. In an animal model of antidepressant-induced anxiolytic effects, the novelty-induced hypophagia (NIH) test, a single administration of the SSRI citalopram reportedly elicited anxiogenic-like effects, whereas three administrations over 24 h were sufficient to produce anxiolytic-like effects. Extending these findings, the present study examined the effects of acute and repeated escitalopram in a similar NIH test in a commonly used mouse strain (i.e., C57BL/6J), analyzing results with a method (i.e., survival analysis) that can model the skewed distribution of latencies to consume food and that can deal with censored data (i.e., when consumption does not occur during the test). Saline-treated mice showed robust novelty-induced hypophagia. Acute escitalopram enhanced novelty-induced hypophagia, but did so only at a dose (i.e., 32 mg/kg) that similarly enhanced hypophagia in a familiar environment. The effects of escitalopram on novelty-induced hypophagia did not significantly change after repeated (3 x) administration over 24 h. Additional studies are necessary to delineate the conditions under which rapid reversal of SSRI-induced anxiety can be modeled in animals using the NIH test.

Keywords

escitalopram; diazepam; anxiety; novelty-induced hypophagia; C57BL/6J mice; Kaplan-Meier survival analysis; log rank (Mantel-Cox) test; mouse

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Introduction

In humans, chronic treatment with selective serotonin reuptake inhibitors (SSRIs) decreases anxiety, unlike acute treatment, which can increase anxiety. Although this biphasic pattern is observed clinically (e.g., Den Boer et al., 1987; Den Boer and Westenberg, 1990; Gorman et al., 1987), preclinical demonstrations are rare (Belzung et al., 2001; Griebel et al., 1994). In an animal model of antidepressant-induced anxiolytic effects, the novelty-induced hypophagia (NIH) test (Dulawa and Hen, 2005), a single administration of the SSRI citalopram reportedly elicited anxiogenic-like effects, whereas anxiolytic-like effects were observed after three administrations over 24 h (Mombereau et al., 2010). In an effort to extend these findings, the present study examined the effects of escitalopram administered acutely or repeatedly over 24 h in a similar NIH test in a commonly used mouse strain (i.e., C57BL/6J), and analyzed the results with a method (i.e., survival analysis) that can model the skewed distribution of latencies to consume food and that can deal with censored data (i.e., when consumption does not occur during the 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 x 18 x 13 cm) with bedding and with free access to food and water. Behavioral testing was conducted in accordance with local IACUC and NIH guidelines; at the time of testing, the median age was 11 weeks (range: 9–15).

Procedure

The NIH test was conducted using a procedure similar to that detailed elsewhere (Dulawa and Hen, 2005). Briefly, mice were singly housed on a Friday and used in the experiment the following week. Monday through Wednesday, they were presented for 10 min each day with diluted, sweetened condensed milk (1:2; Carnation: tap water) in a tube with a stainless-steel drinking spout. Mice that did not consume the milk by Wednesday were eliminated from the experiment (approximately 5% were excluded). Home cage testing occurred on Thursday for 10 min under dim lighting (120 lux). Novel cage testing occurred on Friday for 10 min under bright lighting (1200 lux) in a clean cage, without bedding, placed on white paper. Some mice were tested in the home cage both on Thursday and on Friday. Test sessions were videotaped, and latency to consume the milk was measured in s by an observer unaware of the treatment conditions. Mice were given an injection of saline 30 min before the home cage test, and an injection of saline or escitalopram (3.2, 10, or 32 mg/kg) 30 min before the novel cage test. In the 24 h between these two injections, some mice were given two additional injections of escitalopram, one 30 min after the home cage test and one 5 h before the novel cage test, both at the same dose they were given 30 min before the novel cage test; all other mice were given two additional injections of saline instead. Thus, each mouse received three injections over 24 h between the test on Thursday and the test on

Friday, i.e., three injections of saline, two injections of saline followed by one injection of escitalopram, or three injections of escitalopram at the same dose.

Drugs

Escitalopram (s-citalopram) oxalate (Sigma-Aldrich, St. Louis, MO) was dissolved in physiological saline and injected s.c. at doses expressed as base weight per kilogram body weight. The injection volume was 10 ml/kg.

Data analysis

Because of their non-normal distribution and the occurrence of censoring (i.e., animals that did not consume milk during the 10-min test period), latencies to start milk consumption (defined as licking the drinking spout for three consecutive seconds) were analyzed by Kaplan-Meier survival analysis (e.g., Powell et al., 2012; Guilloux et al., 2013). Differences between groups were examined by the Mantel-Cox log-rank test, and the Holm-Sidak method was used to obtain multiplicity-adjusted p values. To facilitate comparison with previously reported results, latencies were analyzed also by Student's t-test, by one-way ANOVA (i.e., dose) followed by multiple comparisons using Dunnetts' test, and by two-way ANOVA (i.e., dose, acute vs repeated administration of escitalopram) and multiple comparisons by Holm-Sidak. In the latter analyses, censored data were assigned a maximum latency of 600 s. Differences were considered statistically significant when $p < 0.05$. All analyses were performed using Prism 7.03 (GraphPad, San Diego, CA).

Results

Latencies to consume sweetened condensed milk in the home cage during 10 min sessions on Thursdays had a severely skewed distribution (data not shown; median: 34 s; mean: 44 s; skewness: 2.6; kurtosis: 8.2) that deviated significantly ($p < 0.001$) from normality (D'Agostino and Pearson test). Due to their non-normal distribution, data were analyzed by Kaplan-Meier survival analysis. Saline-treated mice showed a significant increase in latency to consume when tested in a novel cage on Friday, compared with saline-treated mice tested in the home cage on Friday (Fig. 1, panel A). The time it took to reach 50% of the mice consuming increased 5.6-fold (95% confidence limits: 2.1 – 15) from 25 s in the home cage to 139 s in the novel cage. This novelty-induced hypophagia was not affected by a single administration of escitalopram at 3.2 or 10 mg/kg, but was significantly increased at 32 mg/kg (Fig. 1, panel B). At this dose, the latency to consume was similarly increased in the home cage (Fig. 1, panel C). Repeated (3 x) administration of escitalopram over 24 h did not significantly alter its effects on latency to consume (Fig. 1, panel D). Similar results were obtained when the latency data were analyzed by t-tests and ANOVA (Fig. 2), i.e., novelty increased the latency to consume (panel A), which was increased further by 32 mg/kg escitalopram (panel B), in a similar manner in the home cage (panel C), and not significantly altered by repeated administration (panel D).

It is worth noting that before starting the present experiments with the NIH test, we examined the anxiolytic diazepam as a positive control. For this, we used male wildtype mice from our serotonin transporter (SERT) mutant colony, created on a C57BL/6J

background (e.g., Mitchell et al., 2016). As expected (e.g., Guilloux et al., 2013), diazepam at 3.2 mg/kg significantly attenuated novelty-induced hypophagia (Fig. 3), confirming the utility of the NIH test to detect anxiolytic drug activity.

Discussion

The present finding that escitalopram enhanced the latency to consume sweetened condensed milk in a novel environment extends previous observations by Mombereau et al. (2010) with citalopram in CREB^α-related wildtype mice. However, in the present study, the latency-increasing effects of escitalopram were not selective for the novel environment, but occurred similarly in the familiar environment. Also, repeated administration of escitalopram over 24 h did not decrease its effects on latency. Because there are many differences between the present experiments and those reported by Mombereau et al. (2010) (e.g., escitalopram vs citalopram, mouse strain, type of palatable food, housing conditions) additional studies are necessary to delineate the conditions under which a rapid reversal of SSRI-induced anxiety can be modeled in animals using the NIH test, or other tests used to detect effects of antidepressants on anxiety (e.g., marble burying; for a review, see Borsini et al., 2002). Among these studies, those examining strain differences may be particularly relevant, given a recent report that chronic fluoxetine could have induced a paradoxical anxiogenic effect in a novelty-suppressed feeding test in C57BL/6J mice, but not in BALB/c mice (Gosselin et al., 2017). Note, however, that fluoxetine has been generally reported to exert anxiolytic activity in the NIH test in both of these mouse strains after having been administered during three or more weeks (Dulawa et al., 2004; Holick et al., 2008; Karlsson et al., 2008; Stan et al., 2015).

The observation that escitalopram increased the latency to feed similarly in the novel and the familiar environment is consistent with a report that citalopram decreased food intake in rats (Grignaschi et al., 1998). Together, these findings suggest the possibility that in the NIH test, the latency-increasing effects of escitalopram reported here, and of citalopram reported elsewhere (Andreasen et al., 2015; Refsgaard et al., 2017), are not necessarily indicative of anxiogenic-like activity.

Here, and in Guilloux et al. (2013), survival analysis and ANOVA had similar outcomes. Future analysis of NIH test results with both survival analyses and ANOVA will help to further evaluate the robustness of ANOVA to deviations of normality and to censoring of NIH data.

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Figure 1.

Effects of escitalopram on novelty-induced hypophagia in adult male C57BL/6J mice. Panel A: Effects of novelty on consumption of sweetened condensed milk. Data are expressed as the cumulative percentage of mice that started consumption during a 10 min session in a novel cage (solid line; $n=11$) and in a familiar cage [home cage (HC); dotted line; $n=7$] (Mantel-Cox log-rank (LR) test: $p<0.001$). Panel B: Escitalopram, administered once, enhanced novelty-induced hypophagia, but only at 32 mg/kg (LR test vs saline: Holm-Sidak adjusted $p=0.0012$) (at 3.2, 10, and 32 mg/kg, $n=7$, 8, and 11, respectively). Saline control data are replotted from panel A. Panel C: 32 mg/kg escitalopram, administered once, increased the latencies to consume similarly (LR: $p=0.76$) in the novel (solid line; $n=11$) and in the familiar cage (dotted line; $n=9$). Novel cage data are replotted from panel B. Panel D: Effects of escitalopram on novelty-induced hypophagia were not significantly affected by frequency of administration (LR tests of acute vs repeated administration, conducted separately at each dose, yielded Holm-Sidak adjusted $p=0.14$) (dashed lines: doses administered 3 times over 24 h; at 3.2, 10, and 32 mg/kg, $n=9$, 10, and 11, respectively) (solid lines: doses administered once, data replotted from panel B).

Figure 2.

Effects of escitalopram on novelty-induced hypophagia, expressed as mean (+SEM) latency to drink, in adult male C57BL/6J mice. Panel A: Consumption of sweetened condensed during a 10 min session in a novel cage (n=11) and in a familiar cage (home cage (HC); n=7) (Student's t-test: p=0.017). Panel B: Escitalopram, administered once, enhanced novelty-induced hypophagia, but only at 32 mg/kg (one-way ANOVA: Dunnett's adjusted p<0.001) (at 3.2, 10, and 32 mg/kg, n=7, 8, and 11, respectively). Saline control data are replotted from panel A. Panel C: 32 mg/kg escitalopram, administered once, increased the latency to consume similarly (Student's t-test: p=0.60) in the novel (n=11) and in the familiar cage (n=9). Novel cage data are replotted from panel B. Panel D: Effects of escitalopram on novelty-induced hypophagia depended on dose, but not on administration frequency (two-way-ANOVA; dose: p<0.001; acute vs repeated administration: p=0.20; interaction: p=0.13) (tests of acute vs repeated administration, conducted separately at each dose, yielded Holm-Sidak adjusted p 0.21) (doses administered 3 times over 24 h: at 3.2, 10, and 32 mg/kg, n=9, 10, and 11, respectively; doses administered once: data replotted from panel B).

Figure 3.

Effects of diazepam on novelty-induced hypophagia in adult male wildtype mice from a serotonin transporter mutant colony, created on a C57BL/6J background (e.g., Mitchell et al., 2016). At 3.2 mg/kg, diazepam (Sigma/RBI, Natick, MA) [dissolved in vehicle consisting of 70% emulphor, 20% sterile water, and 10% ethanol (by volume), and injected i.p., 30 min before testing; all other details as described under Methods] significantly attenuated novelty-induced hypophagia (survival analysis, log rank test: $p=0.035$; figure insert, t-test: $p=0.0043$; $n=8-10$).