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Milnacipran affects mouse impulsive, aggressive, and depressive-like behaviors in a distinct dose-dependent manner

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ABSTRACT

Serotonin/noradrenaline reuptake inhibitors (SNRIs) are widely used for the treatment for major depressive disorder, but these drugs induce several side effects including increased aggression and impulsivity, which are risk factors for substance abuse, criminal involvement, and suicide. To address this issue, milnacipran (0, 3, 10, or 30 mg/kg), an SNRI and antidepressant, was intraperitoneally administered to mice prior to the 3-choice serial reaction time task, resident–intruder test, and forced swimming test to measure impulsive, aggressive, and depressive-like behaviors, respectively. A milnacipran dose of 10 mg/kg suppressed all behaviors, which was accompanied by increased dopamine and serotonin levels in the medial prefrontal cortex (mPFC) but not in the nucleus accumbens (NAc). Although the most effective dose for depressive-like behavior was 30 mg/kg, the highest dose increased aggressive behavior and unaffected impulsive behavior. Increased dopamine levels in the NAc could be responsible for the effects. In addition, the mice basal impulsivity was negatively correlated with the latency to the first agonistic behavior. Thus, the optimal dose range of milnacipran is narrower than previously thought. Finding drugs that increase serotonin and dopamine levels in the mPFC without affecting dopamine levels in the NAc is a potential strategy for developing novel antidepressants.

1. Introduction

Major depressive disorder (MDD) is a major health problem in the world and often leads to suicide.[1] Although selective serotonin reuptake inhibitors (SSRIs) and serotonin/noradrenaline reuptake inhibitors (SNRIs) are widely used for the treatment for MDD, these drugs induce several side effects including increased aggression and impulsivity,[2,3] which are risk factors for substance abuse, criminal involvement, and suicide.[4–8] In contrast, animal studies have shown that SSRIs suppress aggression[9] and that an SNRI and noradrenaline reuptake inhibitor suppress impulsivity.[10–12]

Dose-dependent effects of these drugs might reconcile these contradictory findings because inverted U-shaped dose-response relationships are often observed in psychoactive drugs.[13] Thus, a significant issue concerns whether there is an optimal dose at which an antidepressant suppresses all these behaviors of impulsivity, aggression and depressive symptoms.

There are reliable animal behavioral paradigms that can be used to measure impulsive, aggressive, and depressive-like behaviors, which include the 3-choice serial reaction time task (3-CSRTT),[10] resident-intruder test (RIT),[14] and forced swimming test (FST),[15] respectively. To address above issue, these tests must be conducted in the same animal because food restriction in the 3-CSRTT and isolation in the RIT would alter dose response of drugs.[16,17] Indeed, previous studies have reported that acute milnacipran, an SNRI and antidepressant, reduced the immobility duration in the FST, but the doses at which anti-immobility effects were observed were at least three times higher than those required for anti-impulsive effects in another study.[10,18] However, these behaviors have yet to be examined simultaneously in the same animal study. Therefore, we addressed this issue by using milnacipran and examining the effects on impulsive, aggressive, and depressive-like behaviors in the same animal.

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To further determine the neural mechanisms underlying the effects of milnacipran on impulsive, aggressive, and depressive-like behaviors, we measured dopamine and serotonin levels in the medial prefrontal cortex (mPFC) and nucleus accumbens (NAc) following milnacipran administration by using *in vivo* microdialysis. These brain regions and monoamines have been implicated in the modulation of impulsive,[11,19] aggressive,[20] and depressive-like[21] behaviors. Moreover, noradrenaline transporters take up not only noradrenaline but also dopamine from the extracellular space in brain regions including the mPFC.[22]

Additionally, we examined the correlational relationships between impulsive, aggressive, and depressive-like behaviors to explore the potential overlaps in neural mechanisms underlying these behaviors and to determine whether the interrelationships between these psychological components in humans[23] are preserved in animals.

2. Materials and methods

2.1. Subjects

Male C57BL/6N mice supplied by Nippon SLC Co. Ltd. (Hamamatsu, Japan) and bred in our laboratory were used for the 3-CSRTT, RIT (resident), FST, and microdialysis experiments. Male BALB/c mice supplied by CLEA Japan, Inc. (Tokyo, Japan) were used for the RIT (intruder) experiment. They were housed under an alternating light–dark cycle (lights on from 7 p.m. to 7 a.m.) at approximately 21 °C and a relative humidity of 40–50%. The treatment of animals was in compliance with the Guidelines for the care and use of Laboratory Animals of the Animal Research Committee of Hokkaido University.

2.2. Drugs

Milnacipran hydrochloride was generously donated by Asahi-Kasei Co. Ltd. (Tokyo, Japan). The compound was dissolved in 0.9% saline (pH = 6.5–6.8) and administered at a volume of 10 ml/kg.

2.3. 3-Choice serial reaction time task (3-CSRTT)

When the mice were 9 weeks old, they began individual housing and food-restricted diets. Thereafter, their body weights were maintained at 85% of the body weight of mice under free-feeding conditions.

Training and test procedures in the 3-CSRTT have been described in our previous studies using rats.[10,24,25] Detailed procedure in mice was described in supplementary methods because some steps were modified for mice (Supplementary methods).

We used seven behavioral parameters, as described below.

(a) Premature responses (counts per session): a measure of impulsive action.
(b) Accuracy (percentage of correct responses): a measure of attentional function.
(c) Omissions (counts per session): a measure of attentional function and motivation.
(d) Perseverative responses (counts per session): a measure of compulsive behavior.
(e) Correct response latency (s): a measure of attentional function, motivation, and motor function.
(f) Reward latency (s): a measure of motivation and motor function.
(g) Started trials (counts per session): a measure of motivation.

Eight mice received an acute administration of milnacipran (0, 3, 10, or 30 mg/kg, i.p.) 60 min prior to 3-CSRTT testing. Each drug session was conducted with more than a week interval from the previous drug session. The order of the drug injections was counterbalanced using a Latin square design. All 3-CSRTTs were performed between 9:00 a.m. and 11:00 a.m.

2.4. Resident–intruder test (RIT)

After the mice had been individually housed and had been subjected to 3-CSRTT training for 5–6 weeks, they were studied to measure their aggression toward an intruder (Fig. 1). The RIT was conducted as described in Ref. 26. Detailed procedure was described in supplementary methods (Supplementary methods).

Eight resident mice received an acute administration of milnacipran (0, 3, 10, or 30 mg/kg, i.p.) 60 min prior to an encounter with an intruder. Each drug session was conducted with more than a two-day interval from the previous drug session. The order of the drug injections was counterbalanced using a Latin square design. All RITs were performed between 2:00 p.m. and 5:00 p.m.

2.5. Forced swimming test (FST)

Following the completion of the 3-CSRTT testing, 14 mice were subjected to the FST to measure depressive-like behavior (Fig. 1). Thirty-two naïve mice were used for FST drug testing. They received an acute administration of milnacipran (0, 3, 10, or 30 mg/kg, i.p., n = 8 each) 60 min prior to the FST. The FST was conducted as described in Ref. 27. Detailed procedure was described in supplementary methods (Supplementary methods). We used a between-subject design for the FST to avoid repeated exposure to severe stress. All FSTs were performed between 9:00 a.m. and 11:00 a.m.

2.6. In vivo microdialysis and HPLC analysis

Fifty-six mice were used for the HPLC analysis. Mice were anesthetized with isoflurane (Intervet. Inc., Tokyo, Japan) and fixed in a stereotaxic frame (Narishige, Tokyo, Japan). Guide cannulas (AG-4 for the mPFC or CXG-6 for the NAc, Eicom, Japan) were implanted in either the mPFC [AP: +1.9, ML: ±0.2, DV: −0.6] or the NAc [AP: +1.2, ML: ±0.5, DV: −3.25].[28] After surgery, mice were housed individually and allowed a 1-week recovery period before they began testing.

A dialysis probe (for the mPFC, 2-mm long, A-I-4-02, Eicom; for the NAc, 1-mm long, CX-I-6-01, Eicom) was inserted through the guide cannula. The probe was perfused with artificial CSF (2.7 mM KCl, 140 mM NaCl, 1.2 mM $CaCl_2$, 1.0 mM $MgCl_2$, 0.3 mM NaH_2PO_4, and 1.7 mM Na_2HPO_4, pH 7.2) at a flow rate of 1 μl/min. Mice were placed in plastic observational cages (30 × 30 × 35 cm) and samples were collected every 30 min. Less than 10% variation of the basal monoamine levels was obtained 2–3 h after the insertion of the dialysis probe. Drugs were given by intraperitoneal injection after at least three stable baseline samples were obtained. Dopamine and serotonin concentrations were measured as described previously.[29]

2.7. Data analysis

Behavioral data from the 3-CSRTT and RIT were subjected to a repeated measures analysis of variance (ANOVA) using drug as a within-subject factor. Behavioral data from the FST were subjected to a one-way ANOVA using drug as a between-subject factor. In the microdialysis experiment, the area under the curve (AUC) values for the dopamine and serotonin levels 120 min after the drug

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