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Combined treatment with subchronic lithium and acute intracerebral mirtazapine microinjection into the median raphe nucleus exerted an anxiolytic-like effect synergistically

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ABSTRACT

Although preclinical and clinical studies have established the efficacy of lithium augmentation of antidepressant drugs, the mechanism of action of lithium augmentation is not fully understood. Our previous study reported that subchronic lithium treatment enhanced the anxiolytic-like effect of systemic mirtazapine. In the present study, we examined the effect of subchronic lithium in combination with acute local intracerebral injection of mirtazapine on fear-related behaviors in a contextual fear conditioning test in rats to clarify the target brain region of lithium augmentation of mirtazapine. After conditioning by footshock, diet (food pellets) containing Li_2CO_3 at a concentration of 0.2% was administered for 7 days. Ten min before testing and 7 days after conditioning, mirtazapine (3 μg/site) in a volume of 0.5 μl was acutely injected into the median raphe nucleus (MRN), hippocampus or amygdala. The combination of subchronic lithium and acute mirtazapine microinjection into the MRN but not the hippocampus or the amygdala reduced fear expression synergistically. These results suggest that intra-MRN mirtazapine treatment with subchronic lithium exerts the anxiolytic-like effect through the facilitation of the MRN-5HT pathway.

1. Introduction

Enhancement of serotonergic neurotransmission has been regarded as the primary pharmacological target for the treatment of major psychiatric disorders, particularly those that involve depression and/or anxiety (Graeff et al., 1996). The median raphe nucleus (MRN), similar to the dorsal raphe nucleus, is known to be a major source of serotonin (5-hydroxytryptamine; 5-HT) innervation that sends serotonergic efferents predominantly to the hippocampus and, less extensively, to the amygdala (Vertes et al., 1999; Vertes and Linley, 2007), regions that have been implicated in anxiety and fear (Inoue et al., 2011). Moreover, the serotonergic pathway that goes from the MRN has been proposed to play an important role in contextual conditioned fear (i. e., re-exposure to an environment paired previously with inescapable electric footshock), an animal model of anxiety (Andrade et al., 2013).

Lithium, a mood stabilizer, has been reported to increase the effectiveness of antidepressant drugs clinically, but the mechanism of action of lithium augmentation needs to be further elucidated (Bauer et al., 2014). Animal studies have shown that subchronic lithium increased the anxiolytic-like effect of various serotonergic antidepressants in contextual conditioned fear (An et al., 2015; Kitaichi et al., 2006; Muraki et al., 1999). Further, subchronic lithium treatment not only increases extracellular 5-HT concentrations at baseline in various brain regions but also increases the elevating effect of various antidepressants on extracellular 5-HT concentrations additively, suggesting that the augmentation effect of lithium is mediated by the facilitation of 5-HT neurotransmission (Kitaichi et al., 2004, 2006; Muraki et al., 2001; Wegener et al., 2003). Because a recent study reported that lithium moderates the manic-like behavioral alterations induced by an electrolytic lesion of the MRN in mice (Pezzato et al., 2015), the MRN is a candidate region for lithium augmentation. However, no study to date has investigated the relationship between lithium and the behavioral effect of antidepressants injected into the MRN in the contextual fear conditioning.

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Recently, we reported that the intra-MRN injection of mirtazapine, which increases extracellular 5-HT levels in the hippocampus innervated by the MRN (Yamauchi et al., 2012), reduced fear-related behavior, freezing, in contextual fear conditioning (An et al., 2013) and that subchronic lithium enhanced the anxiolytic-like effect of systemic mirtazapine without affecting general motor activity (An et al., 2015). Accordingly, considering reported evidence on the role of the MRN-5HT pathway and the mechanism of action of lithium on the serotonergic systems, we hypothesized that the behavioral effect induced by the stimulation of serotonergic neurons in the MRN can be increased by subchronic lithium treatment in an animal model of anxiety. Therefore, the study was designed to assess the effectiveness of subchronic lithium with acute local mirtazapine treatment in the MRN, hippocampus and amygdala in rats using the contextual fear conditioning test as an animal model of anxiety. Mirtazapine, which is an α_2-adrenergic antagonist, is considered to stimulate serotonergic neuronal firing through the stimulation of noradrenergic neurons in the MRN and increase the extracellular 5-HT levels in the forebrain (An et al., 2013; Kakui et al., 2009; Millan, 2006; Yamauchi et al., 2012).

2. Materials and Methods

2.1. Animals

A total of 93 male Sprague-Dawley rats (260–320 g) from the Shizuoka Laboratory Animal Center (Shizuoka, Japan) were used. Animals were kept under controlled light (light phase: 06:30–18:30) and temperature (22 ± 2 °C) conditions. Experiments began after a two-week acclimatization period. The animals were maintained on a diet of standard laboratory rat chow or rat chow containing 0.2% of Li_2CO_3 for 7 days. In the lithium experiments, the lithium-treated rats and the control rats had free access to food and 10 mM NaCl instead of tap water to prevent lithium-induced hyponatremia. All procedures were approved by the Hokkaido University School of Medicine Animal Care and Use Committee and were in compliance with the Guide for the Care and Use of Laboratory Animals.

2.2. Drug administration

Bilateral or unilateral infusions of mirtazapine were given with a 33-gauge injector cannula connected by polyethylene tubing to motor-driven microsyringes. Mirtazapine (obtained from Merck & Co. Inc., Whitehouse Station, NJ, U.S.A.) was dissolved at a concentration of 6 μg/μl in 0.15% tartaric acid, and 0.5 μl was infused through each injector at a rate of 0.5 μl/min. The vehicle alone was administered as a control.

The doses of mirtazapine were determined based on our previous experiments in which 3 μg of mirtazapine into the MRN showed the anxiolytic-like effect (An et al., 2013). The concentrations of lithium carbonate in the rat chow were chosen based on our previous studies, in which we observed that plasma lithium levels were 0.71 ± 0.05 mEq/l after 7 days of 0.2% Li_2CO_3 treatment in diet (Muraki et al., 1999). This plasma lithium level is within the clinical therapeutic range (0.5–1.2 mEq/l) (Suppes et al., 2008; Bauer et al., 2010).

2.3. Stereotaxic surgery

Surgeries were performed under sodium pentobarbital (40 mg/kg, intraperitoneally) anesthesia using aseptic conditions. The head position was adjusted to place the bregma and lambda in the same horizontal plane in a stereotaxic frame. Rats were stereotaxically implanted with a unilateral or bilateral 26-gauge stainless steel guide cannula directed toward the MRN (unilateral), amygdala (bilateral, the basal nucleus of the amygdala) or dorsal hippocampus (bilateral) (coordinates of injection sites relative to bregma: AP −7.8 mm, ML +0 mm, V 8.6 mm for the MRN; AP −2.8 mm, ML ±5.0 mm, V 8.4 mm for the amygdala; AP −3.3 mm, ML ±1.9 mm, V 2.9 mm for the dorsal hippocampus; taken from the stereotaxic atlas of Paxinos and Watson (1997)). The guide cannulae for the MRN were unilaterally inserted at a lateral angle of 20° to avoid the sagittal sinus and cerebral aqueductal obstruction. After the surgery, rats were housed individually. When not used for injection, the guide cannulae were occluded with obturators made of 33-gauge stainless steel wire.

2.4. Fear conditioning and behavioral measures

To compare our results with the previous findings (An et al., 2015; Kitaichi et al., 2006), for fear conditioning, the rats were individually subjected to a total of 2.5 min of inescapable electric footshocks (five, 2.5 mA scrambled footshocks, pulse wave, 30 s duration) using a Model SGS-02D Shock Generator (Medical Agent, Kyoto, Japan) in a shock chamber with a grid floor ($19 \times 22 \times 20$ cm, Medical Agent). The shock generator provides a circuit with resistance controlled by dial settings calibrated by the manufacturer in a short circuit current. At the setting of 2.5 mA, this generator delivered 0.2-mA shock intensity to the rats. Seven days after surgery, rats were submitted to footshocks (fear conditioning). Immediately after the footshock, the rats received standard laboratory rat chow (0% Li_2CO_3) or rat chow containing 0.2% of Li_2CO_3 for 7 days until the beginning of the freezing behavior test session.

Seven days after the footshock test, bilateral or unilateral infusions of mirtazapine (3 μg/site, 6 μg/μl) or vehicle (0.5 μl of 0.15% tartaric acid) were performed for 1 min using a 33-gauge injector cannula projecting 1.0 mm beyond the tips of the guide cannula. The solution (0.5 μl) was infused through each injector at a rate of 0.5 μl/min. The injectors were left in place for 60 s after the infusion to allow diffusion and avoid reflux. Ten min later, the rats were again placed in the shock chamber and were observed for 5 min without shocks. During the 5-min observation period, freezing behavior was recorded using a time-sampling procedure, in which the animal behavior was classified as either freezing or as activity at 10-s intervals as previously described (Inoue et al., 2004). Freezing was defined as the complete absence of movement, except movement related to respiration. All other behaviors were scored as activity. The percentage freezing score (freezing (%)) was computed as the proportion of 10-s periods during which the animal remained frozen the entire time.

The exact placement of the injector cannula tips was verified by the injection of Fast Green (2%; 0.5 μl) at the end of the experiments.

2.5. Data analysis

All data are presented as the mean ± S.E.M. of the individual values of the rats from each group. Statistical analysis of the data was conducted using one-way analysis of variance (ANOVA), followed by Bonferroni's test for multiple comparisons as a post hoc test. The significance level was set at 0.05.

3. Results

The injection sites were verified to be localized inside the MRN, hippocampus and amygdala, as illustrated in Fig. 1. In 12 rats, the position of the cannula was located outside of the target area and