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The effects of lobeline on depression-like behavior and hippocampal cell proliferation following chronic stress in mice

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HIGHLIGHTS

- Chronic unpredictable stress (CUS) caused depression-like behavior in mice.
- Lobeline produced antidepressant-like effects in CUS-exposed mice.
- Lobeline increased hippocampal BDNF and cell proliferation in CUS-exposed mice.

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ABSTRACT

We have reported that brain nicotinic acetylcholine receptor (nAChR) ligand lobeline has antidepressant-like effects in mice. The present study examined the effects of lobeline on chronic unpredictable stress (CUS)-induced depression-like behavior, deficits in brain-derived neurotrophic factor (BDNF) expression and cell proliferation in the hippocampus. Adult C57BL/6J mice were exposed to CUS for 6 weeks. Lobeline (1 or 4 mg/kg, s.c.) or saline was administered once daily during the last 14 days of CUS. CUS-exposed mice showed increased immobility time in the FST compared to control. Pretreatment with lobeline (1 mg/kg) significantly reduced immobility time in the CUS-exposed mice. Twenty-four hour following lobeline or saline treatment, BDNF expression or cell proliferation was measured in the hippocampus using Western blotting and bromodeoxyuridine immunohistochemistry, respectively. Lobeline (1 mg/kg) treatment prevented CUS-induced reduction in BDNF expression and cell proliferation in the hippocampus. Overall, our findings suggest that antidepressant-like effects of lobeline could involve nAChR mediated signaling, BDNF expression, and/or hippocampal cell proliferation.

1. Introduction

Stressful life events and chronic stress can precipitate or exacerbate major depression [1]. In animal models, chronic unpredictable stress (CUS) produces depression-like behavior, suppresses brain-derived neurotrophic factor (BDNF) expression and neurogenesis in the adult hippocampus [2,3]. Conversely, chronic administration of some antidepressants increases both BDNF expression [4] and cell proliferation in the hippocampus [5]. Furthermore, disruption of antidepressant-induced cell proliferation by genetic manipulation or X-ray irradiation blocks behavioral responses to antidepressants [6], indicating the critical role of hippocampal neuroplasticity in major depression. More importantly, CUS-induced adverse changes in BDNF expression and hippocampal cell proliferation are prevented by antidepressants [7].

Cholinergic dysfunction is identified as one of the underlying causes of the behavioral deficits following stress [8]. Evidence suggests that chronic stress decreases acetylcholinesterase activity and increases extracellular acetylcholine (ACh) levels in the hippocampus [9,10]. However, it is not known how cholinergic mechanism is associated with chronic stress-induced deficits in hippocampal neuroplasticity. Consistent with an increase in ACh leading to stress, clinical studies showed that blockade of brain nicotinic ACh receptor (nAChR) may improve mood in depressed patients [11,12]. Recently, we have demonstrated that lobeline reduces depression-like behavior as well as ethanol drinking behavior in mice [13–19]. The observations from receptor binding studies suggest that lobeline is a non-selective nAChR antagonist with high affinity for α4β2 and α3β2 nAChRs [20]. We have found that lobeline significantly reduces immobility time in the forced swim test (FST) in mice

Abbreviations: ACh, acetylcholine; BDNF, brain-derived neurotrophic factor; BrdU, 5-bromo-2′-deoxyuridine; CUS, chronic unpredictable stress; FST, forced swim test; HPA, hypothalamic-pituitary-adrenal; nAChR, nicotinic acetylcholine receptor; PFC, prefrontal cortex.

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[13]. In addition, we have reported that lobeline decreases forced swim stress-induced increased norepinephrine and corticosterone levels in mice. The antidepressant-like effects of lobeline are similar to typical nAChR antagonist mecamylamine, suggesting the effects of lobeline could be mediated by blocking nAChR mediated signaling. We have also shown that chronic, but not acute, lobeline treatment decreases feeding latency in the novelty suppressed feeding test, another measure of antidepressant-like effect [13]. Given these findings, we have hypothesized that chronic lobeline treatment would prevent CUS-induced depression-like behavior and deficits of hippocampal neuroplasticity. Therefore, the objective of the present study was to determine the effects of chronic lobeline treatment on CUS-induced depression-like behavior, BDNF expression and cell proliferation in the hippocampus.

2. Materials and methods

2.1. Animals

Male C57BL/6J mice (7–8 weeks old) were obtained from Jackson Laboratory (Bar Harbor, ME) and allowed to acclimatize for one week. Animals were housed in individual cages (29 cm × 18 cm × 12 cm), under standard laboratory conditions (22 ± 2 °C, relative humidity 50-60%) and maintained on a 12/12 h light/dark cycle (lights on at 6:00 AM). Food and water were available *ad libitum* during the experiments unless otherwise specified. All efforts were made to minimize animal suffering and to reduce the number of animals used. All procedures were in compliance with guidelines of National Institutes of Health and were approved by Institutional Animal Care and Use Committee at South Dakota State University.

2.2. Drugs and chemicals

Lobeline and bromo-deoxyuridine (BrdU) were purchased from Sigma–Aldrich (St. Louis, MO). Lobeline (1 or 4 mg/kg, s.c.) and BrdU (150 mg/kg, i.p.) were dissolved in saline before injection in a volume of 0.01 ml/g body weight of animal. Doses were selected based on previous studies [13,21].

2.3. Chronic unpredictable stress

A CUS paradigm was used as an animal model of depression with minor modifications [22]. Total 35 mice were subjected to various and repeated unpredictable mild stressors twice daily for 6 weeks. The stressors included 45° tilted cage (12 h), cage switching (20 min), no bedding, no bedding with water on bottom (250 ml water added into the cage), damp bedding (250 ml water added into the cage), paired housing (2 h), overnight illumination, food deprivation (overnight), and inversion of light/dark cycle. A separate group of 14 unstressed control mice were placed in individual cages for 6 weeks. To mimic a realistic situation of antidepressant intervention, we started drug treatment at the later stage of CUS. Lobeline or saline was injected once daily during the last 14 days of CUS. Twenty-four h after the last injection, mice were subjected to the FST or sacrificed for Western blot/immunohistochemistry.

2.4. Forced swim test

The FST is a widely used behavioral test to assess efficacy of antidepressants in rodents [23]. Mice (n = 5–6/group) were placed individually in a cylindrical Plexiglas tank (45 cm high × 20 cm diameter) that was filled with 25 cm of water (20–22 °C). The time spent immobile during the 15 min test was recorded as described previously [13]. Immobility was counted when no additional activities were observed other than that required keeping the head above water. Mice were removed from the cylinder immediately after the test, dried with paper towel, and kept under a heating lamp until completely dry before returning to their home cages.

2.5. Western blot analysis

To determine the effects of lobeline treatment on BDNF expression, Western blot analysis (n = 4–5/group) was performed as previously described with minor modifications [7]. The prefrontal cortex (PFC) and hippocampus were dissected, frozen in liquid nitrogen, and stored at −80 °C until analysis. Tissue samples (PFC and hippocampus) were homogenized in modified RIPA buffer containing Dulbucco's phosphate-buffered saline (pH 7.4), 1% Igepal CA 630, 0.1% sodium dodecyl sulfate (SDS), and protease inhibitor mix (cOmplete mini, Roche, Indianapolis, IN). The samples were centrifuged (16,000 × g, 20 min at 4 °C) and supernatants were collected. Protein concentration was determined by bicinchoninic acid assay (Pierce, Rockford, IL) using albumin as standard. Equal amounts of protein (60 μg) were loaded onto 10% gels for SDS polyacrylamide gel electrophoresis. Separated proteins were transferred onto nitrocellulose membranes at 80 V for 90 min. Membranes were blocked on a gyro-rocker with 5% nonfat dry milk in Tris-buffered saline/0.1% Tween-20 (TBST) for 1 h, rinsed with TBST, and subsequently incubated overnight at 4 °C with primary antibodies for BDNF (H-117, 1:300, rabbit polyclonal, Santa Cruz Biotech) or β-tubulin (E7, 1:5000, mouse monoclonal, University of Iowa). After incubation, membranes were washed in TBST, followed by incubation with appropriate horseradish peroxide-conjugated secondary antibodies, diluted in blocking buffer at a concentration of 1:5000. Bound antibodies were detected with ECL Prime reagent (Amersham, Buckinghamshire) and protein quantification was performed using densitometric analysis.

2.6. Immunohistochemistry

To assess the effects of lobeline on hippocampal cell proliferation, mice (n = 3/group) were injected with BrdU and sacrificed by decapitation 2 h later [21]. The brains were removed and placed in 4% phosphate-buffered paraformaldehyde solution for 24 h. Post-fixed brains were transferred to 30% sucrose solution in phosphate buffer and stored at 4 °C for at least 48 h. Brains were sliced on a Leica cryostat (40 μm), free-floating sections were washed in phosphate buffer (pH 7.4) and treated with 0.3% hydrogen peroxide to block endogenous peroxidase activity. Brain sections were processed for BrdU labeling. BrdU immunohistochemistry was performed using BrdU in-situ detection kit (BD Biosciences, San Jose, CA) according to manufacturer's instructions. Immunopositive cells in the dentate gyrus of the hippocampus were manually counted under high magnification (x400) by an experimenter unaware of animal groups as previously described [24].

2.7. Statistical analyses

Data were analyzed by one way analysis of variance (ANOVA) followed by Tukey's *post-hoc* test for multiple comparison using GraphPad Prism (GraphPad, San Diego, CA). The difference between treatment was considered statistically significant at $p < 0.05$. All results were expressed as mean ± SEM.

3. Results

The effects of chronic lobeline treatment in the FST are shown in Fig. 1. There was a main effect of treatment on immobility time in the FST ($F_{3,17} = 7$; $p < 0.01$). Multiple comparisons of means revealed that immobility time was significantly higher in CUS-exposed mice compared to control ($p < 0.05$). Conversely, pretreatment