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Antidepressant-like effects of lobeline in mice: Behavioral, neurochemical, and neuroendocrine evidence

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ABSTRACT

Preclinical and clinical studies suggest that neuronal nicotinic acetylcholine receptor (nAChR) antagonists have antidepressant-like properties. The present study examined the effects of lobeline, a nAChR antagonist, in the forced swim test (FST), tail suspension test (TST), and novelty suppressed feeding test (NSFT) of antidepressant efficacy. Lobeline (1 or 4 mg/kg, s.c.) was administered 20 min before the FST and TST in C57BL/6J mice. Pretreatment with lobeline significantly reduced immobility time in the FST but not in the TST. Repeated lobeline (1 or 4 mg/kg, s.c.) treatment for 21 days significantly reduced feeding latency in the NSFT. We also determined the effects of lobeline on forced swim stress (FSS)-induced increased in plasma corticosterone levels using enzyme immunoassay. Pretreatment with lobeline (1 mg/kg, s.c.) significantly attenuated the corticosterone levels. Further, the effects of lobeline on FSS-induced increased in norepinephrine (NE) and serotonin levels in the prefrontal cortex (PFC) and hippocampus were determined using high performance liquid chromatography. Pretreatment with lobeline (1 or 10 mg/kg, s.c.) significantly reduced NE levels in the PFC. Overall, the present study indicates that lobeline produces antidepressant-like effects by targeting brain nAChRs and/or neuroendocrine and brain noradrenergic systems.

1. Introduction

An increased activity of brain cholinergic system might be associated with depressive symptoms in humans (Janowsky et al., 1972). Recent evidence suggests that decreased neuronal nicotinic acetylcholine receptor (nAChR) function may produce antidepressant-like effects (see, for reviews Mineur and Picciotto, 2010; Shytle et al., 2002). For example, mecamylamine, a non-selective nAChR antagonist, had shown efficacy in patients not responding to conventional antidepressant therapy (Dunbar et al., 2007; George et al., 2008). Additionally, mecamylamine was found to decrease symptoms of major depression in patients with Tourette's disorder (Salin-Pascual et al., 2003). Mecamylamine has also produced antidepressant-like effects in mice (Andreasen et al., 2009; Rabenstein et al., 2006). Additional pharmacological and knockout (KO) mice studies suggest that reduced activity at high-affinity α4β2 nAChRs mediates antidepressant-like effects of nAChR ligands (Andreasen and Redrobe, 2009; Andreasen et al., 2009; Mineur et al., 2007; Rabenstein et al., 2006). However, nicotine patch was shown to decrease depressive symptoms in non-smokers (Salin-Pascual et al., 1995) and chronic nicotine administration results in antidepressant-like effects in rodents (Tizabi et al., 1999), indicating an apparent contradiction to the cholinergic hypothesis. This seeming paradoxical phenomenon can be explained by the fact that chronic nicotine desensitizes nAChRs after transient activation, leading to the functional antagonism of nAChRs (Quick and Lester, 2002). Taken together, inhibition rather than activation of nAChRs might have antidepressant-like properties (Andreasen et al., 2009; Mineur and Picciotto, 2010; Shytle et al., 2002).

Although inhibition of nAChRs is necessary for antidepressant-like effects, there are several other possibilities regarding the mechanism of action of nAChR based therapy (Philip et al., 2010). For example, nAChRs are involved in modulating the neuroendocrine function suggesting a role in depression (Okuda et al., 1993). Nonetheless, it is less clear whether interaction between nAChR and hypothalamic–pituitary–adrenal (HPA) axis is necessary for antidepressant-like properties. Previous evidence has demonstrated that large numbers of patients suffering from major depression have HPA axis hyperactivity and depressive symptoms may be reduced, in part, through inhibiting nAChRs associated with stress-induced activation of HPA axis (Shytle et al., 2002). Furthermore, nAChRs are thought to regulate release of monoamine neurotransmitters (Rahman et al., 2008; Tani et al., 1997) which are linked, in part, to the pathophysiology of depression (Ressler and Nemeroff, 2000).

Evidence suggests that lobeline acts as a potent antagonist at brain nAChRs (Dwoskin and Crooks, 2002; Miller et al., 2000). Lobeline inhibits the effects of nicotine in a concentration dependent manner in voltage-clamped *Xenopus* oocytes expressing α4β2 nAChRs (Damaj

Abbreviations: 3-pyr-cyt, 3-(pyridine-3-yl)-cytisine; FSS, forced swim stress; FST, forced swim test; HPA, hypothalamic–pituitary–adrenal; KO, knockout; nAChR, nicotinic acetylcholine receptor; NE, norepinephrine; NSFT, novelty suppressed feeding test; PFC, prefrontal cortex; TST, tail suspension test.

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et al., 1997). Moreover, lobeline blocks nicotine-evoked [^{3}H] norepinephrine release from rat locus coeruleus cells and nicotine-evoked [^{3}H] dopamine overflow from rat striatal slices (Gallardo and Leslie, 1998; Miller et al., 2000). Additionally, lobeline was shown to displace several α4β2 nAChR ligands using *in-vivo* brain tomographic studies (Musachio et al., 1997). Furthermore, effects of lobeline were not altered by nAChR antagonists (Damaj et al., 1997). For example, behavioral effects of lobeline are neither enhanced nor blocked by β2-selective nAChR antagonist DHβE (Damaj et al., 1997). Similarly, behavioral effects of lobeline remained unchanged or not inhibited in presence of non-selective antagonist mecamylamine (Damaj et al., 1997; Stolerman et al., 1995). Overall, lobeline is a non-selective antagonist with high affinity for α4β2 and α3β2 nAChRs (Dwoskin and Crooks, 2002; Parker et al., 1998).

Recently, nAChRs have been targeted for treating alcoholism (Rahman and Prendergast, 2012) and lobeline was found to reduce voluntary ethanol drinking behavior (Bell et al., 2009; Sajja and Rahman, 2011, 2012) and ethanol-induced increased in midbrain dopamine function in rodents (Sajja et al., 2010). Lobeline also has anxiolytic properties (Brioni et al., 1993) and it inhibits the behavioral and neurochemical effects of psychostimulants in animal models (Harrod et al., 2001; Miller et al., 2001). We have also demonstrated the anxiolytic effects of lobeline in mice (Roni and Rahman, 2011). However, the antidepressant-like properties of lobeline have not been investigated thus far. Therefore, we conducted a series of behavioral studies to examine the antidepressant-like properties of lobeline using the forced swim test (FST), the tail suspension test (TST), and the novelty suppressed feeding test (NSFT) in C57BL/6J mice. In addition, we investigated the effects of lobeline on the forced swim stress (FSS)-induced increased in plasma corticosterone levels, an index of HPA axis activity. Finally, we determined the FSS-induced changes in norepinephrine (NE) and serotonin levels to elucidate the monoaminergic correlates of lobeline treatment in mice.

2. Materials and methods

2.1. Animals

Male C57BL/6J mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA). Animals were acclimated in the animal facility for at least one week. Mice were housed in groups of four in standard shoebox cages (29×18×12 cm), under standard laboratory conditions (22±2 °C, relative humidity 50–60%) and maintained on a 12 h light/dark cycle (lights on at 0600 h) with free access to food and water. The behavioral experiments were conducted between 09:00–16:00 h and mice (approximately 3 months old) were allowed to habituate to the testing room for at least 30 min. Counter-balanced design was used to control for any order effects. All procedures were in compliance with the National Institutes of Health guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at South Dakota State University.

2.2. Drug treatment

Lobeline hydrochloride, mecamylamine hydrochloride, dihyhro-beta-erythroidine (DHβE), fluoxetine hydrochloride, imipramine hydrochloride, and hexamethonium chloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). The 3-(pyridine-3 -yl)-cytisine (3-pyr-cyt) was purchased from Tocris Bioscience (Ellisville, MO, USA). Drugs were dissolved in saline before injections in a volume of 10 ml/kg of body weight. All drugs were administered subcutaneously (s.c.) except 3-pyr-cyt and fluoxetine. Both 3-pyr-cyt and fluoxetine were administered intraperitoneally (Mineur et al., 2009) to avoid possible skin irritation by s.c. injection. Lobeline, mecamylamine, 3-pyr-cyt, DHβE, or hexamethonium was injected 20 min before the experiments according to our previous study (Roni and Rahman, 2011). Classic antidepressant fluoxetine or imipramine was injected 30 min prior to experiments as described (Maeng et al., 2008; Mineur et al., 2009) to ensure optimum antidepressant-like effects. The doses were expressed as salt form of the drugs and were selected on the basis of previous animal studies on lobeline (1, 4, or 10 mg/kg) (Sajja and Rahman, 2011); mecamylamine (1 or 3 mg/kg) (Andreasen and Redrobe, 2009); DHβE (3 mg/kg), hexamethonium (5 mg/kg) (Rabenstein et al., 2006); 3-pyr-cyt (0.5 or 1 mg/kg), fluoxetine (10 mg/kg) (Mineur et al., 2009); and imipramine (20 mg/kg) (Maeng et al., 2008). Separate groups of mice were used for the studies and experimenters were blind to treatment for each test.

2.3. Forced swim test

The FST, a widely used behavioral test to assess the efficacy of antidepressants in rodents (Porsolt et al., 1977), was performed with minor modifications. Following saline or drug treatment, each mouse was placed in a cylindrical Plexiglas tank (45 cm high×20 cm diameter) which was filled with 25 cm of water (20–22 °C). The test was conducted for 15 min because C57BL/6J mice were less immobile during the usual 6 min test (Mineur et al., 2007). The experiments were video recorded and immobility time was measured by two skilled observers. Immobility was counted when no additional activities were observed other than that required to keep 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. For repeated treatment procedure, mice were injected once daily for 7 days and tested 24 h after the last injection (Harrod and Van Horn, 2009).

2.4. Tail suspension test

The TST was conducted as described (Steru et al., 1985). After saline or drug treatment each mouse was suspended by the tail from a hook (distance from floor ~45 cm) attached to the TST chamber using adhesive tape (distance from tip of tail ~1 cm). The experiments were video recorded and duration of immobility (sec) in 6 min test session was calculated manually by two expert observers from the video files. Immobility was defined as the absence of leg or body movements. Mice that climbed up on their tails during test session were gently pulled back. Mice were returned to their home cages after the test and the apparatus was cleaned with 70% ethanol solution between tests.

2.5. Novelty suppressed feeding test

The NSFT is used to assess the efficacy of chronic antidepressant treatment in rodents (Dulawa and Hen, 2005). Mice were subjected to the NSFT as described (Mineur et al., 2007). Saline, lobeline (1 or 4 mg/kg) or mecamylamine (1 mg/kg) was administered daily between 9:00 and 11:00 h for 21 days. At the end of treatment, mice were weighed and foods were removed from the cages. Twenty four hours later mice were weighed again and placed at the corner of the testing apparatus that consists of a square Plexiglas chamber (40×40×35 cm) with wood chips bedding. A single regular chow pellet was placed in the center of the chamber on a round paper (9.5 cm diameter). Latency to chew the pellet was recorded for each mouse. Mice were returned to original home cages immediately after the test with a pre-weighed pellet. The pellet was weighed again after 5 min to determine home cage consumption. Water was removed during home cage feeding test in order to restrict feeding from pre-weighed pellet only.