Pharmacology, Biochemistry and Behavior 139 (2015) 1–6

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Effects of lobeline and reboxetine, fluoxetine, or bupropion combination on depression-like behaviors in mice

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ARTICLE INFO

Article history:
Received 2 July 2015
Received in revised form 1 October 2015
Accepted 7 October 2015
Available online 9 October 2015

Keywords:
Nicotinic receptor
Lobeline
Antidepressant
Drug combination
Major depression
Animal model
Forced swim test
Tail suspension test
Mice

ABSTRACT

Evidence suggests that lobeline, a nicotinic acetylcholine receptor ligand, has antidepressant-like properties in mice. The present study investigated the possible additive or synergistic effects of lobeline in combination with commonly used antidepressants, such as reboxetine, fluoxetine, or bupropion, using the tail suspension test (TST) and the forced swim test (FST) in C57BL/6J mice. Reboxetine (5 or 10 mg/kg, i.p.), fluoxetine (5 or 10 mg/kg, i.p.), or bupropion (2 or 4 mg/kg, i.p.) were administered 30 min before TST or FST. A fixed dose of lobeline (1 mg/kg, i.p.) was injected 15 min prior to tests. Co-administration of lobeline and reboxetine, fluoxetine, or bupropion significantly reduced immobility time in the TST and FST in comparison to the antidepressants used alone. The results suggest that lobeline enhanced the effects of reboxetine, fluoxetine, or bupropion in mice. Therefore, lobeline or similar nicotinic receptor ligand may have therapeutic potential as an adjunct for the treatment of major depression.

1. Introduction

Previous studies suggest that combination of brain nicotinic acetylcholine receptor (nAChR) antagonist with commonly used antidepressants could have beneficial effects (Caldarone et al., 2004; George et al., 2008; Popik et al., 2003; Sanberg et al., 2012). For example, preclinical and clinical studies indicate that nAChR antagonist mecamylamine enhances the effects of selective serotonin reuptake inhibitors (SSRIs; George et al., 2008) or amitriptyline, a tricyclic antidepressant (Caldarone et al., 2004). Additional preclinical studies reveal that mecamylamine potentiates the effects of imipramine or citalopram in mice (Popik et al., 2003). In contrast, mecamylamine or dihydro-beta-erythroidine (β2-nAChR subtype-selective antagonist) failed to increase the effects of SSRIs or selective norepinephrine reuptake inhibitor (SNRIs) in a number of studies (Andreasen and Redrobe, 2009; Popik et al., 2003). Overall, the combination effects remain inconclusive and warrant further pharmacological investigation.

We have demonstrated that nAChR ligand lobeline has antidepressant-like effects likely by targeting β2-containing nAChR subtype (Roni and Rahman, 2011, 2013, 2014). These studies indicated that acute or repeated pretreatment with lobeline significantly reduced depression-like behaviors in the forced swim test (FST). Lobeline, a nAChR antagonist, is known to have higher affinity for α4β2 and α3β2 nACR-subtypes (Damaj et al., 1997; Dwoskin and Crooks, 2002; Parker et al., 1998). The ligand was shown to inhibit nicotine-evoked [^{3}H] norepinephrine release from locus ceruleus cells in vitro (Gallardo and Leslie, 1998). In addition, lobeline blocked nicotine-evoked [^{3}H]dopamine overflow from striatal slices of rat and nicotine-evoked ^{86}Rb + efflux from thalamic synaptosomes (Miller et al., 2000). Based on these recent findings, we hypothesized that lobeline would enhance the effects of commonly used antidepressants.

Interestingly, commonly used antidepressants including reboxetine, fluoxetine or bupropion may also block brain nAChRs (Arias et al., 2010; Miller et al., 2002; Slemmer et al., 2000). Reboxetine is a selective NRI with no affinity for serotonergic or dopaminergic receptors. Reboxetine increases extracellular norepinephrine (NE) levels (Page and Lucki, 2002), downregulates β-adrenergic receptors, and blocks brain nAChRs (Miller et al., 2002). Reboxetine is absorbed rapidly and metabolized by liver cytochrome P450 enzymes. Most frequent adverse effects of reboxetine appear to be dose-dependent (Ciraulo et al., 2011). Conversely, fluoxetine selectively inhibit serotonin reuptake transporter of the presynaptic serotonin neurons, resulting in an increased concentration of serotonin around the synapse (Malagié et al., 1995). Fluoxetine may also inhibit brain nAChRs (Arias et al., 2010). Fluoxetine, metabolized by cytochrome P450 enzymes, has active metabolite

Abbreviations: NAChR, Nicotinic acetylcholine receptor; SSRI, selective serotonin reuptake inhibitor; NRI, selective norepinephrine reuptake inhibitor; FST, forced swim test; NSFT, novelty suppressed feeding test; NE, norepinephrine; TST, tail suspension test.

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http://dx.doi.org/10.1016/j.pbb.2015.10.006
0091-3057/

norfluoxetine. Although generally well tolerated, fluoxetine may produce serious adverse effects which can be managed by lowering the dose (Ciraulo et al., 2011).

Among the commonly used antidepressants, bupropion is considered unique because it inhibits both dopamine and NE reuptake. Hydroxybupropion, the active metabolite of bupropion is a NE reuptake inhibitor. Bupropion is also nAChR antagonist (Slemmer et al., 2000) with smoking cessation effects. Bupropion is well tolerated since it has no effects on serotonergic, muscarinic, or histaminergic system (Ciraulo et al., 2011).

We hypothesized that combination of lobeline with other monoaminergic antidepressants will be additive or synergistic due to the distinct mode of actions of two classes of drugs. The purpose of the present study was to investigate whether lobeline would enhance the effects reboxetine, fluoxetine, or bupropion in the tail suspension test (TST) and FST.

2. Materials and methods

2.1. Animals

Male C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME) were acclimated to the vivarium maintained at 22 ± 2 °C and 50–60% relative humidity for at least one week. Mice were housed in groups of four in Plexiglas cages (29 × 18 × 12 cm) and maintained on a 12-h light/dark cycle (lights on at 6 AM) with free access to food and water. At the beginning of experiments, mice were three-months old (22–26 g). The experiments were performed between 9 AM–4 PM. There was one week interval between the TST and the FST. Separate batch of mice was used for the locomotor activity test. Experimenters were unaware of treatment conditions. All procedures were in compliance with guidelines of the National Institutes of Health and were approved by the Institutional Animal Care and Use Committee at South Dakota State University.

2.2. Drugs

Lobeline hydrochloride, fluoxetine hydrochloride, and bupropion hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO). Reboxetine mesylate was purchased from Tocris Bioscience (Ellisville, MO). All drugs were dissolved in saline (0.9% NaCl) before injections. All drug solutions were injected in a volume of 10 mL/kg of body weight. Reboxetine, fluoxetine, or bupropion was administered (i.p.) 30 min before any test. Lobeline was administered (s.c.) 15 min before any test. These doses were expressed as salt form of the drugs and selected based on previous behavioral studies on lobeline (Roni and Rahman, 2013, 2014), bupropion (Bourin et al., 2009), reboxetine, and fluoxetine (Harkin et al., 2004).

2.3. Tail suspension test

The TST was performed as described (Steru et al., 1985; Roni and Rahman, 2013). Each mouse was suspended by the tail from a hook attached to the TST chamber using adhesive tape (distance from tip of tail ~1 cm). The tests were video recorded and immobility time (s) in the 6 min test session was measured by two trained observers from the video files. Immobility was defined as the absence of leg or body movements. Mice were returned to their home cages after the test and the apparatus was cleaned with 70% ethanol solution between tests.

2.4. Forced swim test

The modified version of the FST was performed for the present study (Porsolt et al., 1977). Each mouse was placed in a Plexiglas tank (45 cm high × 20 cm diameter) filled with 25 cm of water (20–22 °C). The test was conducted for 15 min (Roni and Rahman, 2013). The FST was video recorded and later analyzed by two trained observers from the video files to determine the time spent in floating (immobility time). Mice were considered immobile when no additional activities were observed other than that required to keep the head above water.

2.5. Locomotor activity

In order to test for non-specific stimulant effects, spontaneous locomotor activities of mice were measured in a square chamber (40 × 40 × 35 cm). Animals were familiarized with locomotor activity chamber 24 h before the test. Each mouse was placed in the periphery of the cage and allowed to explore freely for 20 min (Roni and Rahman, 2013). All the sessions were recorded by a video camera mounted about 100 cm above the cage and later analyzed with ANY-maze video tracking software (Stoelting Co., Wood Dale, IL) to automatically measure the total distance traveled (m) in the whole area. Since bupropion has no effect on locomotor activity unless used at high dose (10–20 mg/kg) (Redolat et al., 2005), the present study excluded bupropion groups (2 or 4 mg/kg) from locomotor activity test.

2.6. Statistical analyses

The FST and TST data were analyzed by two way ANOVA followed by Bonferroni *post-hoc* test using Prism (GraphPad Software, Inc. San Diego, CA) with two independent factors corresponding to the level of reboxetine/fluoxetine/bupropion and lobeline. Locomotor activity data were analyzed by one way ANOVA followed by Tukey's *post-hoc* test. The difference between treatments was considered significant at $P < 0.05$. Results were expressed as mean ± S.E.M.

3. Results

3.1. Tail suspension test

Fig. 1 shows the effects of combined treatment with reboxetine and lobeline in the TST. There was a significant main effect of lobeline [$F(1,24) = 30$, $P < 0.01$] and reboxetine [$F(2,24) = 79$, $P < 0.01$], but no significant lobeline by reboxetine interaction in the TST [$F(3,24) = 0.03$, P = ns]. Planned comparison of means revealed that reboxetine (5 or 10 mg/kg) significantly reduced TST immobility in the presence of lobeline ($P < 0.05$) when compared with mice treated with reboxetine alone. Planned comparison of means also showed that lobeline alone decreased immobility time.

Fig. 2 displays the effects of combined treatment with fluoxetine and lobeline in the TST. Two way ANOVA revealed significant main effect of

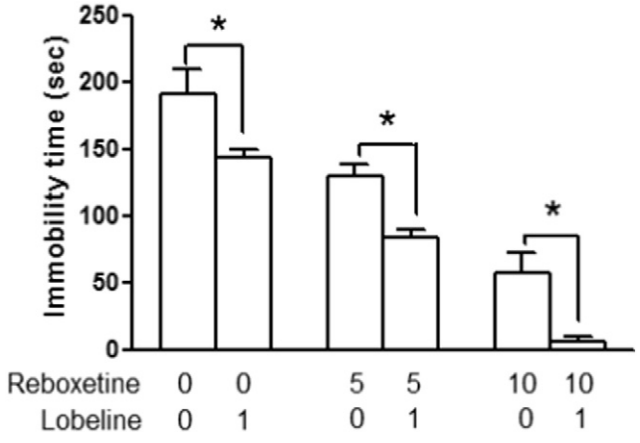

Fig. 1. The effects of reboxetine (5 or 10 mg/kg) were significantly enhanced by lobeline (1 mg/kg) in the tail suspension test (TST). Reboxetine and lobeline were administered 30 and 15 min before the test, respectively. Mice receiving saline treatment were indicated by '0'. Data are expressed as means ± S.E.M. (n = 6–9), *$P < 0.05$.