Behavioural Brain Research 197 (2009) 150–156

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Nicotine, but not mecamylamine, enhances antidepressant-like effects of citalopram and reboxetine in the mouse forced swim and tail suspension tests

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ARTICLE INFO

Article history:
Received 12 July 2008
Received in revised form 12 August 2008
Accepted 16 August 2008
Available online 22 August 2008

Keywords:
Nicotine
Mecamylamine
Antidepressant
Enhancement
Citalopram
Reboxetine
Mouse forced swim test
Mouse tail suspension test

ABSTRACT

Current literature suggests that nicotinic acetylcholine receptors (nAChRs) are involved in major depression. In rodents, antidepressant-like effects of both nicotine and the non-selective nAChR antagonist mecamylamine have been reported. Nicotine increases serotonergic and noradrenergic neuronal activity and facilitates serotonin and noradrenaline release. Thus, we hypothesise that nicotine may enhance the behavioural effects of serotonin (e.g., citalopram) and/or noradrenaline (e.g., reboxetine) reuptake inhibitors. Here, we tested if nicotine enhanced the activity of citalopram or reboxetine in the mouse forced swim test (mFST) and the mouse tail suspension test (mTST). The potential for mecamylamine to augment antidepressant drug action was also investigated. Sub-threshold and threshold doses of citalopram (3 and 10 mg/kg) or reboxetine (3, 10 and 20 mg/kg) were tested alone and in combination with nicotine (0.3 and 1.0 mg/kg) and mecamylamine (1 and 3 mg/kg). Locomotor activity experiments were performed to rule out non-specific stimulant effects. Nicotine (1.0 mg/kg) enhanced the effect of 10 mg/kg citalopram and 20 mg/kg reboxetine in the mFST. Similarly, nicotine (1.0 mg/kg) enhanced the effect of 3 and 10 mg/kg citalopram and 3 and 10 mg/kg reboxetine in the mTST. No concomitant locomotor stimulation was observed at the tested dose combinations. Mecamylamine was effective on its own in some tests, but did not augment the effects of citalopram or reboxetine at the doses tested. The data show that nicotine enhances the effects of both serotonin and noradrenaline reuptake inhibitors, possibly reflecting nicotine's facilitating effects on the release of these two neurotransmitters, and indicating that nicotine may enhance antidepressant action.

1. Introduction

Major depression is among the most debilitating psychiatric disorders with a lifetime prevalence of around 20% [32]. Compounds that inhibit monoamine reuptake and/or metabolism have provided effective treatment for more than four decades [20]. However, despite improved side-effect profiles with the newer selective serotonin (SSRI) and/or noradrenaline reuptake (SNRI/NRI) inhibitors, the relatively slow onset of action and a lack of efficacy in patients with severe depression have generally not been improved [6,42]. Therefore, there is a continuous effort to develop new therapeutic approaches that may reduce time-to-onset, improve efficacy or further improve side-effect profiles.

Clinical findings suggest a link between nicotinic acetylcholine receptors and depression (for review, see Quattrocki et al. [37]). For instance the smoking rate is much higher among depressed patients than among non-depressed individuals [7] and transdermal nicotine patches improve mood in non-smoking depressed patients [27]. Preclinical evidence supports the suggested link between modulation of nicotinic receptors and antidepressant-like effects. While numerous preclinical studies have focused on antidepressant-like effects of nAChR agonists or antagonists *per se* [1,8,9,30,34,38,46,48,49,53,54] few studies have addressed the possible modulation by these compounds of the behavioural responses to standard antidepressant drugs [9,35,36,53].

In the dorsal raphe nucleus (DRN), a brain structure known to be the major source of the forebrain serotonergic innervation, the presence of nAChRs have been demonstrated in mice [26], rats [2,4,5,10,11,15] and humans [3]. In rats, both α4β2 and α7 nAChR have been identified on serotonergic neurons in the DRN [4,5]. In vitro, nicotine stimulates discharge of 60–68% of serotonergic neurons in the DRN and reduces it in the remaining 32–40% [23,28,29]. Similarly, in vivo electrophysiological recordings have shown both stimulatory [17] and inhibitory [13,51] effects of systemic nicotine on serotonergic neurons in the DRN. Noradrenergic neurons in rat locus coeruleus (LC) also contain α4β2 [33,55] and α7 nAChRs [4,55] and these neurons are activated by nicotine in vitro [12] as well as in vivo [18,22,47,51].

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doi:10.1016/j.bbr.2008.08.016

Presynaptic nAChRs have a well-documented role in facilitating transmitter release [41,56]. Specifically, nicotine stimulates release of serotonin (5-HT) [19,25,40,50] and noradrenaline (NE) [31,50] in various forebrain regions. Thus, it is conceivable that nicotine-induced facilitation of 5-HT and NE release may enhance or prolong the behavioural effects of drugs that inhibit 5-HT and/or NE transporters. Indeed, Popik et al. [35] reported enhancements by nicotine of the effects of the tricyclic antidepressant imipramine and the SSRI citalopram in the mTST. The authors also found that mecamylamine enhanced effects of both antidepressants.

The present study was conducted to examine the effects of nicotine administration on the actions of two antidepressants with distinct mechanisms of action, namely the selective SSRI citalopram and the NRI reboxetine. To further the investigation of nicotine's effects on antidepressant-like actions already reported in the mTST, we employed two different behavioural tests for antidepressant action: the mouse forced swim test (mFST) and the mTST. Additionally, locomotor activity studies were performed to ascertain whether the effects observed in the mFST and the mTST were influenced by non-specific stimulant effects, as psychostimulants have been suggested to induce false-positive antidepressant-like actions [43,45].

Considering reports of antidepressant-like actions of mecamylamine in the mFST and mTST [1,9,30,38], the potential for mecamylamine to augment antidepressant drug action was also investigated to directly compare the effects of agonism and antagonism of nAChR on the actions of the two antidepressant drugs.

In the literature, 'enhancements' are often reported when the combined effect of two different treatments are merely additive, i.e. not truly synergistic. In the following, we have employed a strict definition of the term 'enhancement', using this term only when the effect elicited by a combined treatment is supra-additive and significantly different from the effects observed with either treatment alone.

2. Materials and methods

2.1. Animals

Female NMRI mice (25–30 g) obtained from Taconic M&B (Ry, Denmark) were used for all experiments and were 7–9 weeks of age at the time of testing. NMRI mice are derived from an outbred stock, and were thus chosen to better replicate a heterogeneous population. After arrival, mice were allowed a minimum of 7 days acclimatization in Macrolon III cages (20 cm × 40 cm × 18 cm) with 8 mice per cage. All cages were enclosed within a Scantainer (Scanbur A/S, DK). Group housing and isolation from male mice is known to suppress estrus in female mice, i.e. the normal 4–5 day cycling in female mice is prolonged [24,52]. Moreover, under these conditions female mice would be expected to cycle synchronously (Lee–Boot effect) [21]. Food and water was available ad libitum on a 12/12 h light/dark cycle with lights on at 6 a.m. Experiments were performed between 9:00 a.m. and 16.00 p.m. in temperature and humidity-regulated rooms (22–24 °C, relative humidity: 60–70%). Each animal was used only once in a between-subject design. All testing procedures were in accordance with "Principles of Laboratory Animal Care" (NIH publication No. 85-23, revised 1985) and the Danish Animal Experimentation Act.

2.2. Apparatus and procedure

Forced swim test: mice (n = 8–9) were individually placed in a beaker (16 cm in diameter) filled with 20 cm water maintained at 23.5–24.5 °C. Total swim distance during the 6-min test period was automatically recorded by a camera mounted above the cylinders and stored on a computer equipped with the relevant software (Viewpoint, Viewpoint Life Sciences, France).

Tail suspension test: mice (n = 8–10) were suspended by the tail with adhesive tape placed ~1 cm from the tip of the tail. Immobility time during the 6 min period was recorded with an automated electromechanical strain gauge device and stored on a computer equipped with the relevant software (Med Associates Inc., Georgia, USA).

Locomotor activity: mice (n = 6–7) were placed individually in transparent cages (30 cm × 20 cm × 25 cm) and were allowed to habituate for 90 min before the test, which lasted 30 min. The activity chambers were equipped with infrared sensors (6 × 2) arranged along the bottom of each wall of the arena (TSE Systems, Bad Homburg, Germany). Locomotor activity was monitored automatically in the chambers and was measured as the interruption of two consecutive infrared sensors. Interruptions of infrared sensor pairs was detected by a control unit and registered by a computer equipped with the relevant software (ActiMot, TSE Systems, Bad Homburg, Germany).

All experiments were conducted as complete factorial designs with all dose combinations represented.

2.3. Drugs and treatment

The following drugs were used: (−)-nicotine hydrogen di-tartrate (Sigma–Aldrich Denmark), the nicotinic acetylcholine receptor antagonist mecamylamine HCl (Sigma–Aldrich Denmark), the SSRI citalopram HBr (Sigma–Aldrich Denmark) and the NRI reboxetine mesylate hydrate (Sigma–Aldrich Denmark).

Nicotine (0.3 and 1.0 mg/kg) and mecamylamine (1.0 and 3.0 mg/kg) were administered s.c. 15 min prior to testing. Citalopram (3 and 10 mg/kg) and reboxetine (3, 10 and 20 mg/kg) were administered i.p. 30 min prior to testing. The intraperitoneal route was chosen for citalopram and reboxetine, as these compounds may cause local irritation when administered subcutaneously. All drugs were dissolved in saline and given in an injection volume of 10 ml/kg. Each dose is expressed as the free base of the drug.

2.4. Statistical analysis

Differences between groups were analyzed by two-way analysis of variance (ANOVA), with two independent factors corresponding to the level of nicotine/mecamylamine and citalopram/reboxetine, respectively, and followed by Planned Comparisons of the predicted means. As an example, the nicotine + citalopram mFST experiment was analyzed by two-way ANOVA with nicotine and citalopram as two independent factors. Planned Comparisons revealed if citalopram showed significant effects at any of the nicotine dose levels (VEH, 0.3 or 1.0 mg/kg, denoted with stars) and if nicotine showed significant effects at any of the citalopram dose levels (VEH, 3 or 10 mg/kg, denoted with hatches). Differences were considered significant when p values were below 0.05.

3. Results

3.1. Forced swim test

3.1.1. Nicotine + citalopram

Fig. 1a shows the effect of combined treatment with nicotine and citalopram in the forced swim test (mFST). The two-way ANOVA revealed a significant main effect of nicotine ($F2,62 = 3.17$; $p = 0.049$) and of citalopram ($F2,62 = 3.19$; $p = 0.048$) and no significant nicotine by citalopram interaction ($F4,62 = 1.48$; $p = 0.218$). However, Planned Comparisons of means showed that nicotine or citalopram alone did not significantly alter swim distance. Citalopram groups alone are represented by the cluster of three bars to the left, whereas nicotine alone is represented by the three light-grey bars. This principle applies for all graphs throughout. In the presence of 0.3 mg/kg nicotine, citalopram did not induce any significant increase in swim distance. However, in the presence of 1.0 mg/kg nicotine, swim distance was significantly increased by 10 mg/kg citalopram when compared to animals treated with either 1.0 mg/kg nicotine only ($p = 0.003$) or 10 mg/kg citalopram only ($p = 0.004$).

3.1.2. Nicotine + reboxetine

Fig. 1b shows the effect of combined treatment with nicotine and reboxetine in the mFST. There was a significant main effect of nicotine ($F2,62 = 7.02$; $p < 0.002$), no significant main effect of reboxetine ($F2,62 = 2.47$; $p = 0.093$) and a significant nicotine by reboxetine interaction ($F4,62 = 2.82$; $p = 0.032$). Planned Comparisons of means revealed that nicotine or reboxetine alone did not significantly alter swim distance. However, in the presence of 1.0 mg/kg nicotine, swim distance was significantly increased by 20 mg/kg reboxetine when compared to animals treated with either 1.0 mg/kg nicotine only ($p < 0.001$) or 20 mg/kg reboxetine only ($p < 0.001$).