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Baclofen and 2-hydroxysaclofen modify acute hypolocomotive and antinociceptive effects of nicotine

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ABSTRACT

The aim of the present study was to evaluate the possible involvement of $GABA_B$ receptors in nicotine-induced hypolocomotion and antinociceptive effects in mice. Animals were exposed to nicotine only once. Acute nicotine hydrogen tartrate salt (3 mg/kg; subcutaneous, s.c.) administration induced hypolocomotion and antinociceptive responses in the tail-immersion and the hot-plate tests. The effects of pretreatment with either the $GABA_B$ receptor agonist baclofen (1, 2 and 3 mg/kg; intraperitoneal, i.p.) or $GABA_B$ receptor antagonist 2-hydroxysaclofen (0.25, 0.5 and 1 mg/kg; i.p.) were evaluated on these behavioral nicotine responses. The $GABA_B$ receptor agonist, baclofen (3 mg/kg, i.p.) abolished nicotine-induced antinociceptive effects in the tail-immersion and the hot-plate tests, but did not modify nicotine-induced hypolocomotion. In addition, the $GABA_B$ receptor antagonist, 2-hydroxysaclofen (1 mg/kg, i.p.) increased nicotine-induced antinociceptive effects in the tail-immersion and the hot-plate tests, and abolished nicotine-induced hypolocomotion. The present results shed light that the $GABA_B$ receptor has an important role in mediating specific acute nicotine responses such as hypolocomotion and antinociception in mice.

1. Introduction

Nicotine is the main active ingredient in tobacco smoke and plays a major role in tobacco addiction (Dani and Balfour, 2011). In rodents, nicotine produces several behavioral responses, including changes in locomotion, nociception, anxiety, learning, memory, rewarding effects and physical dependence (Berrendero et al., 2010). Regarding locomotion and nociception, nicotine induced hypolocomotion (Berrendero et al., 2005; Castañé et al., 2002; Varani et al., 2012) and antinociceptive responses in the tail-immersion and the hot-plate test (Galeote et al., 2008; Trigo et al., 2009; Varani et al., 2012) in mice. The pharmacological effects of nicotine are mediated by the activation of nicotinic acetylcholine receptors, which are widely distributed through the central nervous system. Neuronal nicotinic acetylcholine receptors are pentameric ligand-gated ion channels, composed of either homomeric or heteromeric combinations of different subunits (α_2–α_{10} and β_2–β_4), which generates a wide diversity of receptors with various electrical and binding properties (Millar and Gotti, 2009). Nicotine promotes the release of diverse neurotransmitters in the central nervous system, such as glutamate, γ-aminobutyric acid (GABA), acetylcholine, dopamine, norepinephrine and serotonin (Picciotto and Corrigall, 2002). We have now focused our interest on GABA, the major inhibitory neurotransmitter in the mammalian central nervous system. This amino acid acts on two classes of receptors: ionotropic $GABA_A$ and $GABA_C$, and metabotropic $GABA_B$ receptors. The $GABA_A$ and $GABA_C$ receptors are located mostly postsynaptically (Barnard et al., 1998), while $GABA_B$ receptors are located both pre and postsynaptically (Bowery et al., 2002). The $GABA_B$ receptors are coupled to G proteins and form a heterodimer of $GABA_{B1}$ and $GABA_{B2}$ subunits, both necessary for $GABA_B$ receptors to be functionally active (Marshall et al., 1999).

$GABA_B$ receptors have been reported to modulate several behavioral responses of nicotine related to its addictive properties (Corrigall et al., 2000; Cousins et al., 2002; Fattore et al., 2002, 2009; Le Foll et al., 2008; Paterson et al., 2004; Paterson, 2009; Varani et al., 2011, 2012, in press; Varani and Balerio, 2012). However, the possible effects that could result from the pharmacological activation or blockade of these receptors on the hypolocomotion and antinociception induced by nicotine remain to be

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clarified. In this study, these two behavioral responses induced by acute nicotine administration were evaluated in mice pretreated with baclofen ($GABA_B$ receptor agonist) or 2-hydroxysaclofen ($GABA_B$ receptor antagonist). Acute nicotine locomotor effects were measured in activity boxes and nicotine antinociceptive responses in the tail-immersion and the hot-plate test.

2. Materials and methods

2.1. Animals

Male Swiss Webster mice obtained from Bioterio Central (Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina) and Charles River (France) weighing 22–24 g were housed five per cage, acclimated to the laboratory conditions according to local regulation (SENASA, 2002) (12-h light: 12-h dark cycle, 21 ± 0.5 °C room temperature, 65 ± 10% humidity) and manipulated for three days prior to the experiment for handling habituation. Food and water were available *ad libitum*. Behavioral tests and animal care were conducted in accordance with the standard ethical guidelines (European Community Guidelines on the Care and Use of Laboratory Animals 86/609/EEC and 2001-486/EEC) and approved by the local ethical committee: CICUAL (Institutional Committee for Care and Use of Laboratory Animals, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina). All experiments were performed with the investigators being blind to the treatment conditions.

2.2. Drugs

(−)-Nicotine hydrogen tartrate salt ([−]-1-methyl-2-[3-pyridil]pyrrolidine) (Sigma Chemical Co., Buenos Aires, Argentina), (±) baclofen (Novartis Argentina S.A.) and 2-hydroxysaclofen (Sigma Chemical Co., Buenos Aires, Argentina) were used in this study. Nicotine and baclofen were dissolved in isotonic (NaCl 0.9%) saline solution, and 2-hydroxysaclofen was dissolved in isotonic (5%) glucose solution immediately before use.

2-hydroxysaclofen (0.25, 0.5 and 1 mg/kg) and baclofen (1, 2 and 3 mg/kg) were administered intraperitoneally (i.p.) 10 and 45 min before nicotine (3 mg/kg), respectively. The doses of 2-hydroxysaclofen and baclofen were based on a previous study from our laboratory (Varani and Balerio, 2012). The dose of nicotine hydrogen tartrate salt (3 mg/kg; subcutaneous, s.c.) was chosen taking into account our previous results (Castañé et al., 2002) and it is reported as nicotine hydrogen tartrate salt (1 mg/kg of nicotine hydrogen tartrate salt equals to 0.35087 mg/kg nicotine free base). All drugs were administered in a volume of 10 ml/kg.

2.3. Locomotor activity

The locomotor responses induced by nicotine hydrogen tartrate salt were evaluated by using small locomotor activity boxes ($9 \times 20 \times 11$ cm^3) (Imetronic, Lyon France) in a low luminosity room (5 lx), and with white noise. Each box contained a line of photocells 2 cm above the floor to measure horizontal movements, and another line located 6 cm above the floor to measure vertical activity (rearing). Mice were individually placed in the boxes 5 min after nicotine hydrogen tartrate salt or saline injection, respectively, and the number of activity counts was recorded for a period of 10 min.

2.4. Antinociceptive responses

Two different nociceptive models where different neural pathways are involved in processing the nociceptive signals, the tail-immersion and the hot-plate test, were used to evaluate the antinociceptive responses induced by nicotine.

Tail-immersion test: The antinociceptive responses were determined as previously reported (Simonin et al., 1998), 15 min after nicotine hydrogen tartrate salt or saline injection. The water temperature was maintained at 50 ± 0.5 °C using a thermo regulated water-circulating pump (Clifton, North Somerset UK). The time to withdraw the tail was determined and a cut-off was set-up at 15 s in order to prevent tissue damage.

Hot-plate test: This test was performed as previously described (Simonin et al., 1998), 16 min after nicotine hydrogen tartrate salt or saline injection. The temperature of the plate (Columbus instruments, Columbus OH USA) was kept at 52 ± 0.5 °C. The nociceptive threshold evaluated was the jumping response. In absence of jumps, a 240 s cut-off was used to prevent tissue damage.

Preliminary experiments with subsequent exposure to the tail-immersion and the hot-plate test showed that the previous tail-immersion exposure did not influence the results obtained in the hot-plate (Castañé et al., 2002; Matthes et al., 1996) test. Each mouse received only one treatment.

2.5. Statistical analysis

The results were analyzed using two-way ANOVA, with NIC (saline or nicotine) and the GABAergic ligand (vehicle or GABAergic ligand) administration as between-subjects factors of variation, followed by corresponding *post-hoc* test when appropriate (Tukey's test).

3. Results

3.1. Baclofen did not modify nicotine-induced hypolocomotion

Nicotine hydrogen tartrate salt (3 mg/kg, sc) similarly decreased vertical and horizontal locomotor activity in animals pre-treated with either baclofen or saline (Fig. 1A and B). Two-way ANOVA calculated for vertical locomotor activity revealed a significant effect of nicotine treatment [$F_{(1,73)}=363.456$, $P<0.001$], without effect of baclofen pre-treatment [$F_{(3,73)}=0.521$, NS] and without interaction between these two factors [$F_{(3,73)}=0.683$, NS]. Subsequent *post-hoc* (Tukey) analysis showed a significant effect of nicotine treatment in saline ($P<0.001$) and baclofen (1, 2 and 3 mg/kg; $P<0.001$) pre-treated mice (Fig. 1A).

Two-way ANOVA calculated for horizontal locomotor activity revealed a significant effect of nicotine treatment [$F_{(1,73)}=394.844$, $P<0.001$], without effect of baclofen pre-treatment [$F_{(3,73)}=2.439$, NS] and without interaction between these two factors [$F_{(3,73)}=1.284$, NS]. Subsequent *post-hoc* (Tukey) analysis showed a significant effect of nicotine treatment in saline ($P<0.001$) and baclofen (1, 2 and 3 mg/kg; $P<0.001$) pre-treated mice (Fig. 1B).

3.2. Baclofen abolished nicotine-induced antinociception

Nicotine hydrogen tartrate salt (3 mg/kg, sc) induced antinociceptive responses in the tail immersion and the hot plate tests (Fig. 2A and B). Baclofen pre-treatment abolished the antinociceptive effect of nicotine in both tests (Fig. 2A and B). In the tail-immersion test, two-way ANOVA revealed a significant effect of nicotine treatment [$F_{(1,71)}=31.799$, $P<0.001$], without effect of baclofen pre-treatment [$F_{(3,71)}=1.933$, NS] and a significant interaction between these two factors [$F_{(1,71)}=8.830$, $P<0.001$]. Subsequent *post-hoc* (Tukey) analysis revealed a significant effect of nicotine treatment in the saline ($P<0.001$) and baclofen (1 mg/kg; $P<0.001$) pre-treated mice, but not in mice pre-treated with 2 and 3 mg/kg of baclofen. *Post-hoc* also showed a significant blockade in the antinociceptive response induced by nicotine in the baclofen (3 mg/kg; $P<0.001$) pre-treated animals, but not in mice pre-treated with 1 and 2 mg/kg of baclofen (Fig. 2A).