Available online at www.sciencedirect.com
ScienceDirect
Neuroscience Letters 413 (2007) 68–71
Neuroscience Letters
www.elsevier.com/locate/neulet

Disconnection between activation and desensitization of autonomic nicotinic receptors by nicotine and cotinine

Jerry J. Buccafusco [a,b,*], Laura C. Shuster [a,b], Alvin V. Terry Jr. [a]

[a] *Department of Pharmacology and Toxicology, Alzheimer's Research Center, Medical College of Georgia, Augusta, GA 30912-2300, United States*
[b] *Veterans Administration Medical Center, Augusta, GA 30904, United States*

Received 24 May 2006; received in revised form 2 November 2006; accepted 13 November 2006

Abstract

Cotinine is the major metabolite of nicotine in humans, and the substance greatly outlasts the presence of nicotine in the body. Recently, cotinine has been shown to exert pharmacological properties of its own that include potential cognition enhancement, anti-psychotic activity, and cytoprotection. Since the metabolite is generally less potent than nicotine in vivo, we considered whether part of cotinine's efficacy could be related to a reduced ability to desensitize nicotinic receptors as compared with nicotine. Rats freely moving in their home cages were instrumented to allow ongoing measurement of mean arterial blood pressure (MAP). The ganglionic stimulant dimethylphenylpiperazinium (DMPP) maximally increased MAP by 25 mmHg. Slow (20 min) i.v. infusion of nicotine (0.25–1 μmol) produced no change in resting MAP, but the pressor response to subsequent injection of DMPP was significantly attenuated in a dose-dependent manner by up to 51%. Pre-infusion of equivalent doses of cotinine produced the same maximal degree of inhibition of the response to DMPP. Discrete i.v. injections of nicotine also produced a dose dependent increase in MAP of up to 43 mmHg after the highest tolerated dose. In contrast, injection of cotinine produced no significant change in MAP up to 13 times the highest dose of nicotine. These results illustrate the disconnection between nicotinic receptor activation and receptor desensitization, and they suggest that cotinine's pharmacological actions are either mediated through partial desensitization, or through non-ganglionic subtypes of nicotinic receptors.

Keywords: Acetylcholine receptors; Desensitization; Ganglionic nicotinic receptors; Mean arterial blood pressure; Nicotine metabolites

Cotinine is a primary metabolite of nicotine that can exert measurable effects on certain types of behavior, working memory and cognition. The metabolite enjoys a long pharmacological half-life (15–19 h) relative to nicotine (2–3 h), and in clinical trials, cotinine was reported to be relatively safe at doses up to 10 times that usually obtained during cigarette smoking [10]. In binding studies cotinine was shown to be about 100-fold less potent than nicotine displacing [^{3}H]epibatidine [17]. Data derived from functional assays indicated that cotinine exhibits the properties of a weak α7 subtype nicotinic agonist, but with the ability to desensitize human α7 nicotinic receptors expressed in a non-neural cell line [2,3]. The ability of cotinine to partially desensitize nicotinic receptors was confirmed in studies with adrenal chomaffin cells [17] and with brain vascular endothelial cells [1]. The latter effect included α7 homo-oligomeric and β2-containing heteromeric nicotinic receptor subtypes.

Recently, we reported that cotinine [16] exhibited pharmacological and potential therapeutic properties that were similar in many respects to those produced by nicotinic receptor agonists [4]. For example, in the rat, the motor response to acoustic startle can be inhibited by the presentation of a low-level acoustic prepulse presented just in advance of the high-level acoustic pulse. Many drugs with antipsychotic actions reverse the disruption of acoustic startle by the dopamine receptor agonist apomorphine. Treatment with cotinine significantly reversed the effects of apomorphine on acoustic startle. We also demonstrated that cotinine increased accuracy in an automated delayed matching-to-sample (DMTS) task by monkeys; cotinine was equally effective, but at least 500-fold less potent than nicotine. Lastly, cotinine was shown effective in preventing the cytotoxicity associated with growth factor withdrawal in differentiated PC-12 cells. In this regard, cotinine is slightly more potent than nicotine. PC-12 cells express the ganglionic nicotinic α3- and β2-containing subunits, and the α7 subtype of nicotinic receptors [9]. The purpose of this

* Corresponding author at: Department of Pharmacology and Toxicology, Alzheimer's Research Center, Medical College of Georgia, Augusta, GA 30912-2300, United States. Tel.: +1 706 721 6355; fax: +1 706 721 9861.
E-mail address: jbuccafu@mcg.edu (J.J. Buccafusco).

0304-3940/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2006.11.028

study was to compare the ability of both nicotine and cotinine to activate and then desensitize ganglionic nicotinic receptors in vivo. The ganglionic nicotinic stimulant DMPP was used as the test probe for quantifying desensitization, and the paradigm utilized the slow pre-infusion of nicotine or cotinine followed by the DMPP challenge. This approach paralleled the in vitro method used to assess the ability of drugs to desensitize nicotine-induced rubidium efflux [14].

Male Wistar rats, weighing 250–275 g at the time of the experiment were obtained from Harlan, Sprague–Dawley (Indianapolis, IN). The animals were housed in our institutional animal care facilities for at least 1 week prior to experiments. Food (Harlan Teklad rodent diet, Madison, WI) and tap water were supplied on an unlimited basis. A 12-h light:12-h dark cycle was maintained. Rats had AAALAC-approved toy objects in their cages to promote behavioral well-being. All experimental protocols, consistent with AAALAC guidelines, had been reviewed and approved by the Medical College of Georgia Institutional Animal Care and Use Committee.

Rats were anesthetized initially with sodium methohexital, 65 mg/kg, i.p. and supplemented as needed. A midline abdominal incision was made to expose the iliac artery, and a catheter (PE 50) filled with heparinized (30 units/ml) saline was inserted so that the tip of the catheter terminated in the base of the abdominal aorta below the origin of the renal arteries. The opposite end of the catheter was tunneled under the skin to emerge at the nape of the neck where it was passed through a subcutaneous plastic anchor button and a spring support that was connected to a water-tight swivel cannula mounted above the center of the cage. The catheter was constantly infused with heparinized saline (30 units/ml) to maintain the potency of the catheter. Next an i.v. line filled with heparinized saline was inserted into the left jugular vein. This catheter was also passed through the spring support but was allowed to emerge at a point in the spring support above the animal's reach. Just prior to the experiment the arterial catheter was connected to a pressure transducer coupled to a hard copy device (Western Graftec 8 channel thermal array recorder) and the analog signals were amplified and digitized on a Buxco Electronics LS-14 Logging Analyzer. Stable levels of mean arterial pressure (MAP) were recorded for at least 15 min prior to the start of the experiment. The analyzer was set to provide 10 s cumulative averages for the first 2 min after injection; 1 min averages were continued for the next 3 min; and 5 min averages were continued for the remaining 25 min. The continuous analog signal was captured on the chart recorder.

Each animal was used in a dose–response series for either nicotine or cotinine. Infusions were spaced at least 24 h apart. Data were analyzed by use of a multi-factorial analysis of variance (ANOVA) with repeated measures. Only the first 5 min of data accumulated after DMPP was injected were subjected to statistical analysis as this time period constituted >90% of the pressor response. Error values denoted by ± indicate the standard error of the mean. Differences between means from experimental groups were considered significant at the $P < 0.05$ level (two-sided test).

Baseline (pre-vehicle) MAP was 108 ± 1.9 mmHg. Vehicle (sterile normal saline) was infused at the rate of 25 μl/min for

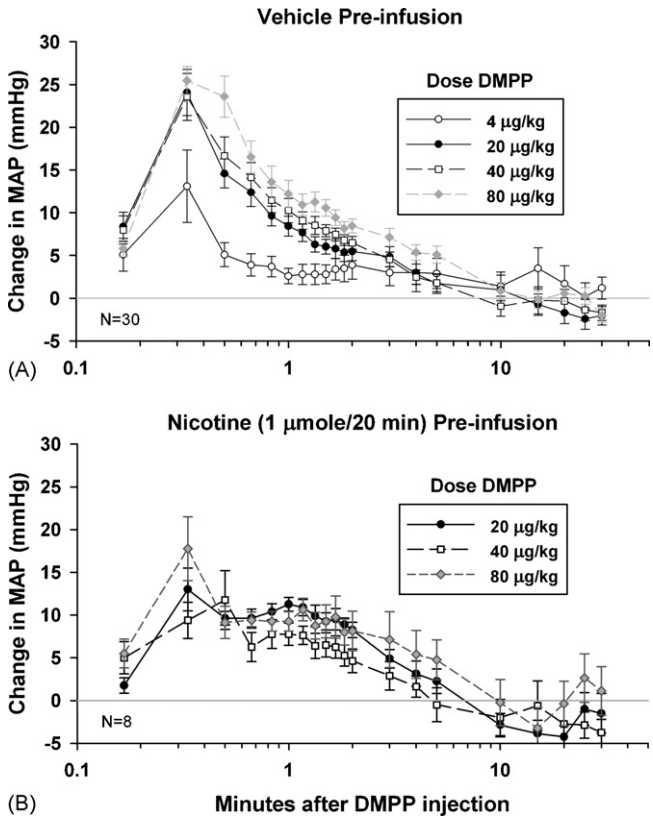

Fig. 1. The increase in mean arterial blood pressure (MAP) produced by i.v. injection of several doses of DMPP in awake rats pre-infused with vehicle (sterile saline) (A) or with 1 μmol nicotine (B) over 20 min as a function of time (note the log scale to better show early time points) after DMPP injection.

20 min. Five minutes after the infusion (the time prior to a challenge dose of DMPP) resting MAP was still 108 ± 1.9 mmHg. The first series of experiments (Fig. 1A) was designed to determine an appropriate test dose of DMPP. Bolus i.v. injection of DMPP produced a dose-dependent increase in MAP up to about 25 mmHg. Pre-infusion (20 min) of 1 μmol of nicotine produced no significant effect on resting MAP (before nicotine = 112 ± 2.4 mmHg; just prior to the probe dose of DMPP = 114 ± 2.2 mmHg), but it decreased the magnitude of the pressor response to subsequent injection of 20, 40, and 80 μg/kg DMPP (Fig. 1B). The 40 μg/kg dose of DMPP was selected as the probe dose because it was the highest dose that evoked no behavioral effect in the rats, and because the effect was sensitive to nicotine pre-infusion.

In the next series, several doses of nicotine were infused prior to the test dose of DMPP. Prior to nicotine infusion the MAP was 117 ± 1.5 mmHg; just prior to DMPP the resting MAP was 113 ± 1.3 mmHg. As shown in Fig. 2A, nicotine infusion from 0.25 to 1.25 μmol produced a dose-dependent inhibition of the pressor response to the test dose of DMPP ($F_{4,525} = 28.4$, $P < 0.0001$). The pressor response following vehicle, was significantly different from each of the responses following nicotine pretreatment ($t > 4.8$, $P < 0.0001$). Higher doses of nicotine were not used because they produced behavioral effects in the animals that made blood pressure measurement unreliable. Cotinine infusion up to 2.5 μmol,