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Enhancement effects of nicotine on neurogenic relaxation responses in the corpus cavernosum in rabbits: The role of nicotinic acetylcholine receptor subtypes

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ABSTRACT

Nicotine acts as an agonist of nicotinic acetylcholine receptors, which belong to a superfamily of neurotransmitter-gated ion channels. We previously demonstrated that nicotine increases the electrical field stimulation (EFS)-evoked nitrergic relaxation responses via activation of nicotinic acetylcholine receptors. The aim of the present study is to investigate the subtypes of nicotinic acetylcholine receptors in rabbit corpus cavernosum. EFS-evoked relaxation responses were recorded from corpus cavernosum strips obtained from rabbits with an isometric force displacement transducers. Effects of nicotine on EFS-evoked relaxations were examined in pre-contracted tissues. Then the effect of nicotine on the EFS-evoked relaxations was examined in the presence of hexamethonium, dihydro-β-erythroidine, mecamylamine or α-bungarotoxin. In our study, nicotine (3×10^{-5}, 10^{-4}) transiently increased nitrergic relaxations induced by EFS in the rabbit isolated corpus cavernosum. While hexamethonium and mecamylamine near totally inhibited or abolished the neurorelaxation response to nicotine (3×10^{-5}) on EFS, dihydro-β-erythroidine and α-bungarotoxin partially inhibited these responses. These findings demonstrated that the alpha3–beta4, alpha4–beta2 and alpha7 subunits of nicotinic acetylcholine receptors play role on the nicotine-induced augmentation in EFS-evoked relaxation responses in rabbit corpus cavernosum.

1. Introduction

Nicotine is an alkaloid derived from the plant *Nicotiana tobaccum*. Nicotine acts as an agonist of nicotinic acetylcholine receptors, which belong to a superfamily of neurotransmitter-gated ion channels. These receptors are located in the central nervous system (CNS) or peripheral nervous system (Newman et al., 2002; McGehee et al., 1995; Todorov et al., 1991). Molecular biology studies have revealed nicotinic acetylcholine receptor subunits. These receptors are composed of nine types of α subunit (α2–α10) and three types of β subunit (β2–β4) subunits (Corriveau and Berg, 1993; Mandelzys et al., 1995; Wonnacott, 1997; Vetter et al., 2007).

Nicotinic acetylcholine receptors modulate synaptic neurotransmission (Newman et al., 2002; McGehee et al., 1995; Todorov et al., 1991). We previously demonstrated that nicotinic acetylcholine receptors are probably located on the nitrergic nerves of the corpus cavernosum tissue in the rabbit. Nicotine seems to act on these nicotinic acetylcholine receptors, thereby evoking the release of nitric oxide (NO), and causing the NO-dependent relaxation of smooth muscle (Bozkurt et al., 2007). In different studies we demonstrated that nicotine increased electrical field stimulation (EFS)-evoked contractile responses (possibly by facilitating neurotransmitter release from nerve terminals) in the gastric fundus, bladder and myometrium of the rabbit (Vural et al., 2006; Nas et al., 2007; Ilhan et al., 2007; Ilhan et al., 2008). The effect of nicotine was dependent on the influx of Ca^{2+} from voltage-gated Ca^{2+} channels via activation of nicotinic acetylcholine receptors in the isolated gastric fundus. We recently showed that the α3β4 and α4β2 subunits of nicotinic acetylcholine receptors have a role in nicotine-induced augmentation of EFS-evoked contractile responses in the gastric fundus in rabbits (Vural et al., 2009).

In the present study, we aimed to characterize the nicotinic acetylcholine receptor subtypes involved in nicotine-induced EFS-evoked alternation of the non-adrenergic non-cholinergic (NANC) relaxation response in the rabbit corpus cavernosum.

2. Materials and methods

2.1. Animals and tissue preparation

A total of twelve New Zealand albino male adult rabbits (4–6 months old; 2.5–3.5 kg body weight) were used for this study. The animals were fed standard laboratory chow and given tap water ad libitum. This study was approved by the Gazi University Ethics Committee for Animals (G.U.ET-09.030). Rabbits were sacrificed by injecting an overdose of thiopental sodium intravenously (50 mg/kg). Penises were rapidly excised and connective tissues, urethra and corpus spongiosum were carefully dissected. The corpora cavernosa were then cut longitudinally into four equal strips ($3\times3\times15$ mm) and were studied separately.

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2.2. Organ chamber experiments

Each strip was mounted under 2 g isometric resting tension in an organ bath containing 15 ml of Krebs–Henseleit solution (composition in mmol/l: NaCl, 118; KCl, 4.7; $CaCl_2{\cdot}2H_2O$, 1.3; $MgCl_2{\cdot}6H_2O$, 0.5; $Na_2HPO_4{\cdot}2H_2O$, 0.9; $NaHCO_3$ 24.9; and glucose monohydrate, 11). The pH of the solution was 7.4 after bubbling with 95% O_2/5% CO_2, and the solution was maintained at 37 °C. Tissues were allowed to equilibrate for at least 1 h before the experimental procedures. EFS-evoked responses were recorded by a Grass isometric force displacement transducers (FT 03, Grass Instruments, Quincy, MA, USA) connected to an ink writing oscillograph (Grass 79 E) via a preamplifier. After a stabilization period, strips were pre-incubated for 30 min with guanethidine (10^{-6}M) and atropine (10^{-6}M) to eliminate adrenergic and cholinergic nerve mediated responses. Then strips were pre-contracted submaximally using phenylephrine (10^{-5}M) until the contraction reached a plateau. Isometric relaxations were evoked by EFS through a platinum electrode, every 2 min, with 4 Hz of stimulation frequencies, trains of impulses of 1 ms duration for 10 s and with a voltage of 60 V. Stimuli were generated by a Grass S48 stimulator. To test the effects of nicotine, different concentrations (3×10^{-5}M, 10^{-4}M) of nicotine were administered to the preparations. EFS were stopped after five relaxations, and the preparations were washed for an hour in every 15 min. Following washing, strips were pre-incubated again for 30 min with guanethidine (10^{-6}M) and atropine (10^{-6}M). Then strips were pre-contracted with phenylephrine (10^{-5}M). After contraction reached a plateau, EFS was delivered again and the same experimental procedure was performed with the same tissue in the presence of hexamethonium (a nonspecific nicotinic acetylcholine receptor antagonist; 10^{-5}M, $n=8$), dihydro-β-erythroidine (α4β2 nicotinic acetylcholine receptor antagonist; 10^{-5}M, $n=8$), mecamylamine (α3β4 nicotinic acetylcholine receptor antagonist; 10^{-5}M, $n=8$) or α-bungarotoxin (α7 nicotinic acetylcholine receptor antagonist; 3×10^{-7}M, $n=8$). Antagonists were added to the organ baths 30 min before the administration of nicotine. Effects of antagonists were examined only nicotine's 3×10^{-5}M concentration.

2.3. Drugs

All drugs (phenylephrine hydrochloride, atropine sulfate, guanethidine monosulfate, nicotine, hexamethonium hydrochloride, dihydro-β-erythroidine, mecamylamine, α-bungarotoxin) were obtained from Sigma (St Louis, MO, USA). Stock solutions of drugs were dissolved in distilled water. Solutions were stored at −20°C until use. The drugs were diluted in Krebs solution to the required final concentration on the day of use.

2.4. Statistics

Nicotine-induced increases were expressed as percentages of the control and the maximum of five EFS-evoked relaxation responses. The value of the last relaxation before the application of nicotine was taken as the control value.

Experimental values were expressed as the mean ± S.E.M. Groups were compared statistically using general linear models of analysis of variance (ANOVA) followed by *post hoc* analysis with the Bonferroni test.

P values of <0.05 were considered to be statistically significant.

3. Results

EFS-evoked relaxation responses in the presence of guanethidine (10^{-6}M) and atropine (10^{-6}M) in the rabbit corpus cavernosum. The mean amplitude of the EFS-evoked NANC relaxation responses was 1.30 ± 0.17 g at a stimulation frequency of 4 Hz.

3.1. Effects of nicotine on neurogenic relaxations of rabbit gastric corpus cavernosum strips

Nicotine increased the EFS-induced non-adrenergic non-cholinergic (NANC) relaxation responses transiently (3×10^{-5}M, 62.66 ± 10.15%; $P<0.05$; 10^{-4}M, 63.83 ± 13.99%; $P<0.05$). Nicotine-induced enhancements were reproducible and were not significantly changed during the second period of EFS after washing. No tachyphylaxis was observed.

Nicotine (3×10^{-5}M) had no relaxation effect on non-stimulated preparations which were pre-contracted with phenylephrine. At 10^{-4}M concentration nicotine-induced relaxation response (18.08 ± 4.34%) on non-stimulated preparations which were pre-contracted with phenylephrine. Nicotine had no effect on preparations maintained only under basal tonus at 3×10^{-5}M and 10^{-4}M concentrations.

3.2. Effects of antagonists on nicotine-induced EFS-evoked transient neurogenic relaxations

Hexamethonium (percentage of inhibition: 97.78 ± 0.85%; $P<0.05$) and mecamylamine (percentage of inhibition: 92.89 ± 1.16%; $P<0.05$) near totally inhibited or abolished nicotine-induced EFS-evoked NANC relaxation response enhancement. Dihydro-β-erythroidine (percentage of inhibition: 47.09 ± 9.76%; $P<0.05$) and α-bungarotoxin (percentage of inhibition: 65.45 ± 5.41%; $P<0.05$) partially inhibited the nicotine-induced responses (Fig. 1).

Hexamethonium, dihydro-β-erythroidine, mecamylamin and α-bungarotoxin had no significant effect on EFS-evoked NANC relaxation responses (Fig. 2).

4. Discussion

In the present study, nicotine transiently increased EFS-induced NANC relaxation responses at concentrations of 3×10^{-5}M and 10^{-4}M. In our previous studies, nicotine increased EFS-induced responses transiently in the corpus cavernosum, gastric fundus, bladder and myometrium of rabbits (Vural et al., 2006; Bozkurt et al., 2007; Nas et al., 2007; Vural et al., 2009). In the present study, a statistically significant difference between the effects of two concentrations of nicotine was not observed. Bagcivan et al. (2004) demonstrated that nicotine-induced concentration-dependent relaxation responses in corpus cavernosum strips in the rabbit when the tissues were pre-contracted with phenylephrine. In that study, authors observed a relaxation response at 10^{-4}M nicotine (similar to our present study). This concentration did not induce significant relaxation, so we used a nicotine dose of 3×10^{-5}M for investigating the effects of antagonists in our experiments (similar to our previous study). We used only one concentration of each antagonist to characterize the nicotinic acetylcholine receptor subtypes involved in the alternation of the nicotine-induced EFS-evoked non-adrenergic non-cholinergic (NANC) relaxation response. We did not aim to find the pA_2 values of antagonists in the present study. Hexamethonium and mecamylamine nearly totally inhibited or abolished the neurorelaxation response to nicotine on EFS, and dihydro-β-erythroidine and α-bungarotoxin partially inhibited these responses. In a previous study, we demonstrated for the first time that nicotine potentiated the relaxation response induced by EFS in strips of corpus cavernosum tissues in the rabbit (Bozkurt et al., 2007). In that study, nicotine-induced potentiation of the EFS relaxation response was abolished by treatment with hexamethonium chloride and tetrodotoxin. Those findings suggested the involvement of neuronal nicotinic acetylcholine receptors in corpus cavernosum tissue in the rabbit.

Relaxation of the smooth muscle of the corpus cavernosum and cavernous artery is critical for inducing and maintaining penile erection (Andersson and Wagner, 1995; Saenz de Tejada, 2002). It was previously demonstrated that relaxation of the corporal smooth muscle is mainly mediated by the nitrergic component of NANC neurotransmission. Noradrenalin is the main factor that leads to