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Hydrogen peroxide and antioxidizing enzymes involved in modulation of transient facilitatory effects of nicotine on neurogenic contractile responses in rat gastric fundus

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Abstract

Nicotine acts as an agonist of nicotinic acetylcholine receptors. Nicotinic acetylcholine receptors play a role in the modulation of neurotransmitter release in both the central and the peripheral nervous system. Moderate reactive oxygen species levels modulate the regulation of physiological functions e.g. neurotransmitter release. Previously in rabbit gastric fundus we demonstrated that nicotine transiently increased neurogenic contraction induced by electrical field stimulation (EFS). In this study we aimed to investigate the effects of hydrogen peroxide (H_2O_2), antioxidizing enzymes catalase and superoxide dismutase (SOD) on nicotine induced increases at cholinergic neurotransmission in rabbit gastric fundus. Although H_2O_2 did not alter nicotine induced transient neurogenic contractions at concentrations of 10^{-6} and 10^{-5} M, at high concentration (10^{-4} M) H_2O_2 inhibited nicotine induced increases. Catalase (500 units/ml), enhanced the effect of nicotine but did not alter nicotine induced transient neurogenic contractions at the concentrations of 100 and 250 units/ml. SOD (75,150 and 225 units/ml) did not alter nicotine induced transient neurogenic contractions. In conclusion, at high concentration H_2O_2 (10^{-4} M) inhibited nicotine's transient ability to augment neurogenic contractions and catalase (500 units/ml) enhanced the effect of nicotine.
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Keywords: Nicotine; Rabbit gastric fundus; Cholinergic neurotransmission; Nicotinic acetylcholine receptor; Hydrogen peroxide (H_2O_2); Catalase; Superoxide dismutase (SOD)

1. Introduction

Nicotine, an alkaloid isolated from the leaves of tobacco plant, is the nonspecific agonist of nicotinic acetylcholine receptors. Nicotinic acetylcholine receptors belong to a super-family of pentameric ligand-gated ion channels and have seventeen subunits identified as of the present time. These receptors are located in both the central and peripheral nervous system (Newman et al., 2002; McGehee et al., 1995; Todorov et al., 1991). Previously, different research groups demonstrated that, noradrenalin release is increased by nicotine via peripheral nicotinic acetylcholine receptors in various tissues such as guinea pig vas deferens, rat stomach, adrenal gland and anococcygeus muscle (Todorov et al., 1991; Nedergaard and Schrold, 1977; Yokotani et al., 2002, 2000; Rand and Li, 1992). Similarly, nicotine modulates acetylcholine release via nicotinic acetylcholine receptors in aganglionic vas deferens and guinea pig ileum myenteric plexus (Cuprian et al., 2005; Briggs and Cooper, 1982). Nicotine triggers influx of Ca^{+2} through ligand-gated channels and/or voltage-gated Ca^{2+} channels (VGCC) via activation of nicotinic acetylcholine receptors. Then the release

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of Ca^{2+} from intracellular calcium stores is triggered. Ca^{+2} activation plays an important role in the action of nicotine via activation of the presynaptic nicotinic acetylcholine receptors and as a result neurotransmitter release increases (Brain et al., 2001; Dolezal et al., 1998; Shoop et al., 2001; Sharma and Vijayaraghavan, 2001).

There are numerous systems that produce reactive oxygen species (hydrogen peroxide, H_2O_2; superoxideradical, O_2-; hydroxyl radical, OH.) in the organism. Reactive oxygen species are responsible for several biological phenomena including carcinogenesis, ischemia-reperfusion injury, radiation damage and neurodegenerative diseases (Toyokuni,1999; Richter et al., 1995). But it was shown that moderate levels of reactive oxygen species modulate the regulation of critical physiological functions, e.g. signaling cascades, control of gene expression (Nose, 2000). It has been demonstrated that H_2O_2 is involved in the regulation of vascular tone. In the rat aorta, H_2O_2 induces contractile responses by increasing intracellular Ca^{2+} levels and this effect is completely reduced by catalase (Yang et al., 1998). Also in smooth muscles it was shown that voltage-dependent Ca^{2+} entry is potentiated by H_2O_2 (Oba et al., 1998). As a result in several studies it was suggested that reactive oxygen species may modulate intracellular Ca^{2+} concentration. Although effects of reactive oxygen species on synaptic neurotransmission was investigated by different research groups previously, this relationship remains poorly understood (Pellmar, 1987; Chen et al., 2001; Vural et al., 2006). Previously in rabbit bladder we demonstrated that reactive oxygen species do not play a physiological role on the regulation of nicotinic acetylcholine receptors dependant neurotransmitter releases (Vural et al., 2006).

In our previous studies, we demonstrated that nicotine increases the electrical field stimulation (EFS)-evoked contractile response, possibly by facilitating neurotransmitter release from nerve terminals by a mechanism dependant on the influx of Ca^{2+} from VGCCs via activation of nicotinic acetylcholine receptors in isolated rabbit bladder, corpus cavernosum and gastric fundus. NO and prostaglandins do not have a physiological role on the regulation of neurotransmitter release in both rabbit gastric fundus and bladder (Bozkurt et al., 2007; Vural et al., 2007; Ilhan et al., 2007). In this study we aimed to investigate the effects of hydrogen peroxide (H_2O_2) and the antioxidizing enzymes catalase and superoxide dismutase on nicotine induced increases in nerve evoked contraction in rabbit gastric fundus.

2. Materıals and methods

2.1. Animals

Twenty New Zealand albino rabbits weighing 2.5–3.0 kg were used for the experiments. All animals were kept under controlled temperature (23.2 °C) and humidity (55.5%) with a 14-h light and 10-h dark cycle. They were fed standard laboratory chow and given tap water. All experiments were performed in accordance with the ethical regulations of the Helsinki Declaration. This study was approved by the Gazi University Ethics Committee for Animals.

2.2. Tissues

Animals were sacrificed by exsanguination and their stomachs were rapidly excised, opened lengthwise, and emptied. Adherent fat, gross connective tissues, and gastric mucosa were removed, and uniform longitudinal strips (15 mm × 3 mm) were prepared from the smooth muscle of the gastric fundus.

2.3. Organ chamber experiments

Each strip was mounted under 1 g isometric resting tension in an organ bath containing 15 mL Krebs–Henseleit solution (composition in mmol/L: NaCl 118.0, KCl 4.7, $CaCl_2.2H_2O$ 1.3, $MgCl_2.6H_2O$ 0.5, $Na_2HPO_4.2\ H_2O$ 0.9, $NaHCO_3$ 24.9, glucose monohydrate 11.0). The pH of the solution was 7.4 after bubbling with a mixture of 95% O_2 and 5% CO_2, and the solution was maintained at 37 °C. The tissues were allowed to equilibrate for at least 1 h before experimental procedures. Isometric contractions were evoked by EFS through a pair of platinum electrodes with an 8 Hz stimulation frequency by 10 s trains of impulses delivered every 2 min. A stimulator (S48; Grass Instruments, Quincy, MA, USA) delivered 60 V pulses of 1 ms duration. EFS-evoked responses were recorded via Grass isometric force displacement transducers (Grass FT 03) connected to an ink writing oscillograph (Grass 79 E) via a preamplifier. Thirty minutes after the EFS-evoked responses reached a steady state, to test the contribution of the cholinergic component, the tissues were treated with atropine (10^{-6} M), non-selective competitive antagonist of muscarinic receptor, or neostigmine (10^{-5} M), a reversible anticholinesterase drug. The effects of tetrodotoxin (3×10^{-6} M), H_2O_2 (10^{-6}–10^{-4} M), catalase (100 units/ml, 250 units/ml and 500 units/ml) or superoxide dismutase (SOD) (75,150 and 225 units/ml) on the EFS-evoked responses were tested similarly in separate strips. The tissues were exposed with drugs at least 30 min. To test the effects of nicotine, different concentrations (3×10^{-5} M, 10^{-4} M) of nicotine were administered to the separate preparations. To avoid any possible habituation effect or tachyphylaxis, EFS was stopped after seven contractions and the preparations were washed four times every 15 min for 1 h as in our previous study (Ilhan et al., 2007). To investigate the effects of reactive oxygen species on the nicotine induced EFS-evoked contractile response alternations, same experimental procedure was repeated in the presence of H_2O_2 (10^{-6}–10^{-4} M), catalase (100 units/ml, 250 units/ml and 500 units/ml) or superoxide dismutase (SOD) (75,150 and 225 units/ml). H_2O_2 and enzymes were added to the organ baths 30 min before the administration of nicotine.

2.4. Drugs

All of the following drugs were obtained from Sigma (St. Louis, MO, USA): nicotine, atropine sulfate, superoxide dismutase (EC 1.15.1.1) from bovine liver, catalase (EC 1.11.1.6) from bovine liver, tetrodotoxin except hydrogen peroxide which was obtained from Merck Schuchardt. Stock solutions of drugs were dissolved in distilled water. Solutions were stored at −20 °C.

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