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Activation of muscarinic receptors inhibits neurogenic nitric oxide in the corpus cavernosum

A.M. Senbel*, A. Hashad, F.M. Sharabi, T.T. Daabees

Department of Pharmacology & Toxicology, Faculty of Pharmacy, Alexandria University, Egypt

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ABSTRACT

The functional role of cholinergic transmission in erection is still far from being fully elucidated. This work aims to further elucidate the modulatory role of neostigmine on NO in the corpus cavernosum and to highlight whether cholinergic transmission in the penis modulates sildenafil action. The isolated rabbit corpus cavernosum and measurement of intracavernosal pressure in the anesthetized rat model were used. Neostigmine (0.02 mg/kg) reduced increase of intracavernosal pressure/mean arterial pressure (ICP/MAP) next to cavernous nerve stimulation. Higher doses (0.06 and 0.4 mg/kg) potentiated ICP/MAP rise and atropine (1.5 and 10 mg/kg) did the opposite. In vitro, neostigmine (10^{-5} and 10^{-4} M) potentiated neurogenic relaxations and this effect was significantly inhibited by hexamethonium (10^{-4} M) or N^{ω}-propyl-L-arginine (3×10^{-5} M) and partially but significantly reduced in the presence of atropine. Lower dose neostigmine (10^{-7} M), inhibited electrically induced relaxation over the range of 1–4 Hz, atropine (10^{-6} M) almost abolished this inhibitory effect as well as N^{G}-nitro-L-arginine (10^{-5} M). It was also significantly reduced by selective nNOS inhibitor N^{ω}-propyl-L-arginine (3×10^{-5} M). Nicotine (10^{-4} M) significantly potentiated electrically induced relaxations amounting to $84.625 \pm 8.06\%$ at 1 Hz and potentiated the effect of sildenafil synergistically. Hexamthonium did the opposite. The potentiatory effect of sildenafil on neurogenic erection was significantly reduced by low dose neostigmine both in vitro and in vivo. This study provides evidence that muscarinic receptors may modulate NO synthesis in nitrergic nerves by inhibiting nNOS and high level of cholinergic stimulation may activate nicotinic receptors to promote erection probably by potentiating NO synthesis in nitrergic nerves.

1. Introduction

Nitric oxide (NO) released from both nitrergic nerve terminals and endothelial cells in the penis activates a NO-sensitive guanylyl cyclase to increase conversion of GTP to cGMP. Cyclic GMP binds with high affinity to cGMP-dependent protein kinase and activates this enzyme to cause phosphorylation of numerous cellular proteins that are involved in modulating the level of cellular calcium and calcium-regulated pathways [1]. Although NO/cGMP is now accepted as the major pathway mediating erection, other physiological pathways are involved such as adrenergic and cholinergic mechanisms and some reports demonstrated potential interaction between these variable pathways [2]. Phosphodiesterase inhibitors play a critical role in the modulation of second messenger signaling pathways by inhibiting hydrolysis of cAMP and cGMP [3]. Phosphodiesterase 5 is the target of three potent PDE5-selective inhibitors (sildenafil, vardenafil and tadalafil), which are available as oral treatment for erectile dysfunction [4]. Some phosphodiesterase inhibitors such as papaverine and theophylline inhibit excitatory enteric neural pathways by depressing synaptic transmission [5]. Moreover, in human vas deferens, sildenafil was found to inhibit adrenergic neurotransmission, an effect not related to the accumulation of cGMP [6]. It is suggested that NO acts presynaptically to facilitate vagal neurotransmission by increasing presynaptic calcium influx and vesicular release of acetylcholine [7]. The detailed characterization of the mechanisms underlying neurotransmitter release and modulation in the penis is of great importance in understanding the functional link between synaptic transmission and phosphodiesterase inhibition in erection and is far from being fully established.

Although cholinergic innervation has been shown in the penile corpus cavernosum, its complete functional role has not been elucidated [8]. ACh released from these nerves acts on muscarinic receptors located on cavernosal smooth muscle and endothelium [9]. Several investigators confirmed the endothelium-dependent action ACh; removing the endothelium reduced relaxant responses to ACh in corporal tissue [10]. On the other hand, parasympathetic

* Corresponding author at: Department of Pharmacology & Toxicology, Faculty of Pharmacy, Alexandria University, Azarita, P.O. Box 21521, Egypt.
Tel.: +20 1006971669; fax: +20 34873273.
E-mail addresses: senbelamira@yahoo.com, senbelamira@alexpharmacy.edu.eg (A.M. Senbel).

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activity may produce penile tumescence and erection by inhibiting the release of noradrenaline through stimulation of muscarinic receptors on adrenergic nerve terminals, and/or by releasing NO and vasodilating peptides from nerves and endothelium [11]. However, in an earlier study, Okamura et al. demonstrated that acetylcholine liberates NO from the endothelium in canine corpora cavernosa but does not act as a neurotransmitter or neuromodulator in inhibitory nerves [12].

This study was designed to investigate the effect of cholinergic modifier on erection and on sildenafil's action in the corpus cavernosum. The major target of the study is to further elucidate the role of cholinergic transmission in erection and its modulatory role on NO in the corpus cavernosum as well as to highlight whether cholinergic transmission in the penis modulates sildenafil action. This potential modulation is of interest in further understanding the detailed mechanism of penile erection, and in suggesting better management for erectile dysfunction.

2. Methods

Experiments were carried out on male albino Wistar rats weighing 250–300 g and white New Zealand rabbits weighing 2.5–3 kg. The animals were obtained from the Alexandria Faculty of Pharmacy Animal House. The research experiment protocol adhered strictly to the Principles of Laboratory Animal Care.

2.1. Measurement of intracavernosal pressure in rats

Experiments have been conducted by measuring intracavernosal pressure (ICP) changes elicited by electrical stimulation of

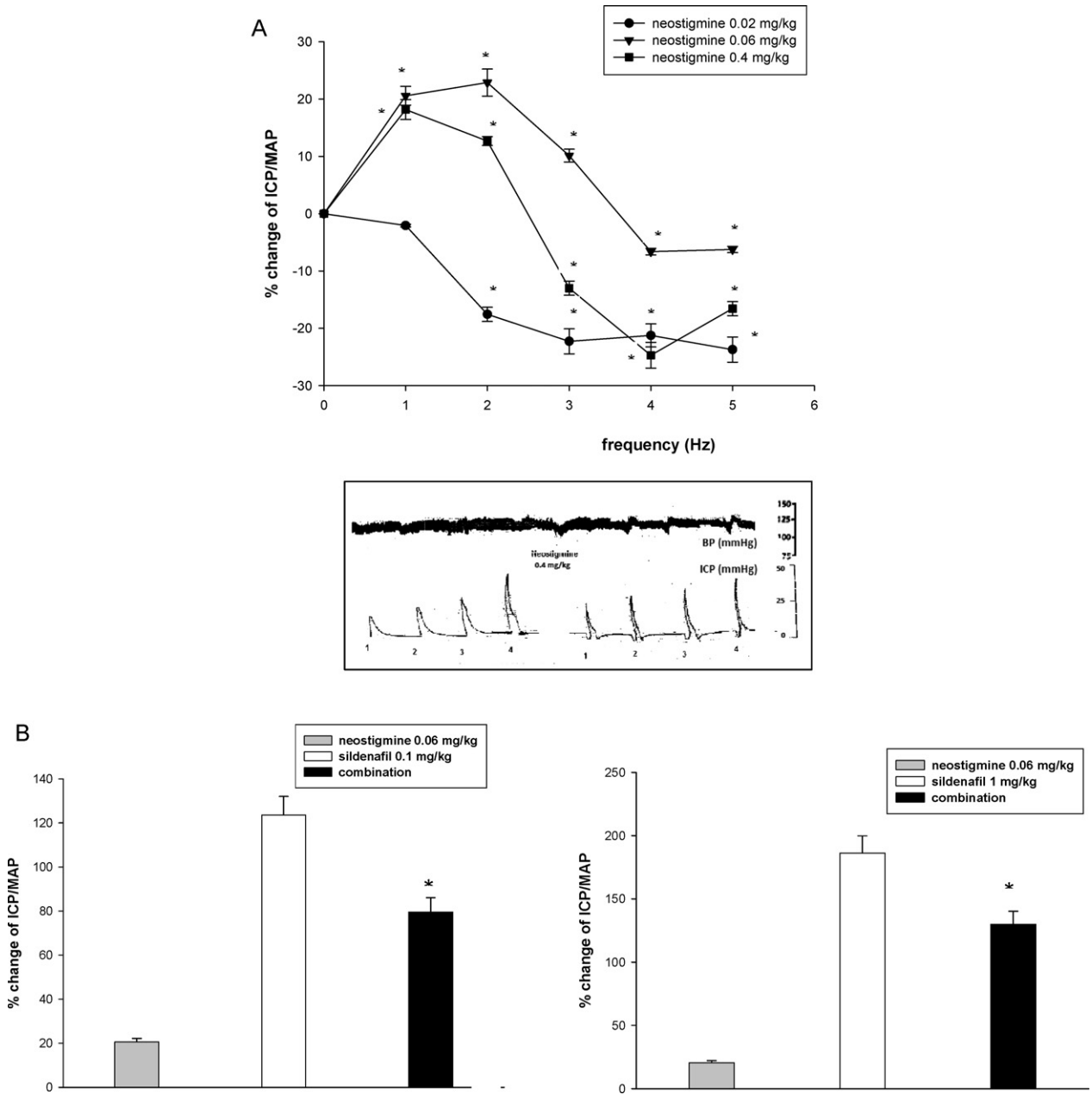

Fig. 1. (A) Frequency response curve of neostigmine (0.02, 0.06 and 0.4 mg/kg) on intracavernosal pressure/mean arterial pressure (ICP/MAP) after being injected intravenously in male rats. Results are expressed as mean ± SEM. *Significant difference than control at the level of $p < 0.05$. Lower panel represents a typical tracing showing the effect of neostigmine (0.4 mg/kg) on ICP and MAP. (B) The effect of sildenafil (0.1–1 mg/kg) in the presence and in the absence of neostigmine (0.06 mg/kg) on intracavernosal pressure/mean arterial pressure (ICP/MAP) at 1 Hz. Results are expressed as mean ± SEM. *Significant difference than sildenafil group at the level of $p < 0.05$.