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Effect of Sildenafil and Rolipram on Adrenergic Responses in Isolated Human and Monkey Corpus Cavernosum

Lang-Chu Lau, P. Ganesan Adaikan *

Department of Obstetrics and Gynaecology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

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Abstract

Objective: Evaluate and compare effects of phosphodiesterase inhibitors (PDEIs), sildenafil and rolipram, on adrenergic contractile responses of human and monkey cavernosal smooth muscle.
Methods: Human penises were obtained from patients undergoing gender reassignment surgery. Isolated human and monkey corpus cavernosum (CC) strips were suspended in tissue bath chambers for isometric tension experiments. The effects of the drugs on precontracted monkey and human CC and neurogenic contractions in human CC were investigated.
Results: Both sildenafil and rolipram induced concentration-dependent relaxations of human and monkey CC strips precontracted with noradrenaline (NA). The IC_{50} values, determined by reverse regression for nitroglycerin (NTG), isoprenaline, and sildenafil in monkey CC, were, respectively, $1.5 \pm 0.9 \times 10^{-7}$ M, $3.7 \pm 0.6 \times 10^{-6}$ M, and $1.7 \pm 0.7 \times 10^{-5}$ M. Similarly, in human CC muscle, sildenafil was weaker than NTG as a muscle relaxant. Sildenafil, 1.5 μM, reduced neurogenic contractions in human CC due to stimulation of predominantly adrenergic nerves. The suppressant effect of sildenafil on adrenergic transmission was attenuated in CC strips pretreated with Nω-nitro-L-arginine and overcome with a higher stimulus frequency or tetraethylammonium. Rolipram partially inhibited adrenergic excitatory response but without significantly affecting NA-induced contraction.
Conclusions: Sildenafil and rolipram induced concentration-dependent reversal of human and monkey CC tone mediated by NA. Both PDEIs attenuated contractile adrenergic response of human CC to electrical stimulation. The results also underline the importance of the cyclic adenosine monophosphate-dependent signalling pathway in regulating the tone. PDE4 inhibition in CC is an additional mechanism for erection and potential therapeutic adjunct.

* Corresponding author. Department of Obstetrics and Gynaecology, National University Hospital, 5 Lower Kent Ridge Road, Singapore 119074, Singapore. Tel. +65 6772 4128; Fax: +65 6779 4753.
E-mail address: obgadaik@nus.edu.sg (P.G. Adaikan).

1. Introduction

Penile erection results from relaxation of both vascular and trabecular smooth muscle in the corpus cavernosum (CC), which allows increased blood flow and engorgement of the lacunar spaces. This smooth muscle relaxation is mediated by nitric oxide (NO) released during sexual stimulation by parasympathetic neurons in the penis and also by the endothelial cells lining the blood vessels and the lacunar spaces of the CC. NO activates soluble guanylate cyclase leading to an increased conversion of guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP), which provides the signal for smooth muscle relaxation. The intracellular level of cGMP is regulated by a balance between its synthesis by guanylate cyclase and its degradation by cyclic nucleotide phosphodiesterases (PDEs). Recent studies have identified at least 11 PDE families (PDE1–11) with distinct tissue distribution, substrate specificity, and drug sensitivity. With respect to substrate specificity, PDE4, 7, and 8 are relatively specific for cyclic adenosine monophosphate (cAMP), whereas PDE5, 6, and 9 are relatively specific for cGMP and the remaining PDEs are of mixed specificity [1]. In CC tissue, the expressions of PDE2, 3, 4, 5, and 11 have been demonstrated and PDE5 is the predominant isoenzyme [2–4]. Inhibitors of PDE5 enzyme such as sildenafil, tadalafil, and vardenafil enhance NO-induced relaxation of CC smooth muscle and facilitate penile erection by increasing the intracellular cGMP level.

Rolipram, a relatively selective inhibitor of cAMP-specific PDE4 isoenzyme, has been reported to relax rabbit and human erectile tissues [5–8]. It also produces a myriad of biologic effects including attenuation of clinical depression and inflammation in the central nervous system. These effects are consequences of rolipram-induced elevation of intracellular cAMP that increases synthesis and release of noradrenaline (NA), enhancing the central noradrenergic transmission and suppressing the expression of proinflammatory cytokines and other mediators of inflammation [9]. PDE4 inhibitors such as cilomilast and roflumilast are potential anti-inflammatory agents for the treatment of diseases such as asthma, chronic obstructive pulmonary disease, and multiple sclerosis [10].

Several putative transmitters and modulators are implicated in the contraction of penile erectile tissues. However, NA and endothelins appear to play a pivotal role in maintaining the penis in a flaccid, non-erected state [11,12]. That the inhibition of sympathetic tone is essentially conducive to penile erection forms the basis underlying the pharmacotherapy for erectile dysfunction using α-blockers such as phentolamine and prazosin. Prostaglandin E_1, which acts via the cAMP pathway, also inhibits adrenergic neurotransmission in the cavernosum [13]. Endogenous inhibition of adrenergic tone has been suggested as a step in the mechanism of penile erection [14]. In vitro studies on human and rabbit CC strips indicated the likelihood of sympathetic responses in these tissues being controlled by nitrergic innervation [15]. Based on these observations, this study was designed to investigate the effects of sildenafil and rolipram on NA-induced tone and adrenergic (excitatory) transmission.

2. Materials and methods

Four adult monkeys weighing 5–8 kg (*Macaque fascicularis*) were euthanised with sodium pentobarbitone followed immediately by penotomy. Human penises were obtained from five patients who underwent male-to-female reassignment surgery. These studies were conducted in accordance with the Policy Governing the Use and Care of Animals in Research and Teaching and the Ethical Committee of National University of Singapore.

Each specimen was collected in oxygenated chilled Krebs–Henseleit solution (composition in mM: NaCl 115, KCl 4.7, $CaCl_2$ 1.8, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 8.5, $NaHCO_3$ 22.1, and EDTA 0.03). The CC smooth muscle was dissected free from the tunica albuginea and used for functional studies within 24 h of collection. A total of 36 human (6–8 strips/specimen) and 16 monkey CC strips (4 strips/specimen) were studied.

Isolated CC preparations (monkey: $1 \times 1 \times 5$ mm; human: $2 \times 2 \times 5$ mm) were mounted in 25-ml organ baths containing Krebs–Henseleit solution maintained at 37 °C, pH 7.4, and aerated with 95% O_2 and 5% CO_2. An initial resting tension of each strip was set at 10–15 mN and the tissues were allowed to equilibrate for 60–90 min. Isometric responses of the tissue were amplified and digitalised with an analog-digital converter (MacLab; ADInstruments, Australia) and recorded for further evaluation and statistical analysis.

Amputated penises from sex change operations offer an important source of human CC tissues for basic research on the mechanisms of penile erection. Earlier studies indicated that these human CC strips, despite exposure to abnormal levels of oestrogens, behaved pharmacologically in a similar manner as CC strips obtained from the macroscopically normal region of penises that were amputated due to penile tumour [11].

2.1. *Cumulative relaxant response curve of PDE inhibitors on monkey/human CC muscle*

Monkey/human CC strips were contracted with 1 μM NA. When stable contraction plateaus had been reached, PDE inhibitors (PDEIs) were added to the organ baths in cumulative concentrations. Maximal relaxant activity could not be attained by rolipram because higher stock solutions were