ORIGINAL RESEARCH—BASIC SCIENCE

Effects of Phenylephrine on Spontaneous Activity and L-Type Ca^{2+} Current in Isolated Corpus Cavernosum Myocytes

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DOI: 10.1111/j.1743-6109.2012.02847.x

ABSTRACT

Introduction. Norepinephrine is important in maintaining detumescent tone in the corpus cavernosum, although the mechanism is incompletely understood. As α-adrenoceptor-induced tone is antagonized by L-type Ca^{2+} channel blockers, it is usually assumed that direct modulation of this current is involved. However, the effects of α-adrenoceptor agonists have never been directly examined on L-type current in corpus cavernosum myocytes (CCSMC), leaving open other possibilities. In particular, CCSMC are now known to develop spontaneous tone via a pacemaker mechanism involving spontaneous Ca^{2+} waves that activate Cl^- currents, causing depolarization and voltage-dependent activation of L-type channels. We hypothesized that phenylephrine modulates tone via this system, rather than by directly activating L-type channels.

Aims. Examine in freshly isolated CCSMC the effect of phenylephrine on: (i) spontaneous Cl^- currents and depolarizations; (ii) cytosolic Ca^{2+} waves; and (iii) L-type current.

Methods. CCSMC were enzymatically dispersed from male New Zealand White rabbits for patch clamp recording and real time Ca^{2+} imaging.

Main Outcome Measures. Spontaneous Cl^- currents, spontaneous depolarizations, cytosolic Ca^{2+} and L-type current.

Results. Phenylephrine enhanced the amplitude and frequency of spontaneous Cl^- currents, increased the duration and frequency of spontaneous depolarizations and increased the frequency of spontaneous Ca^{2+} waves. These effects were blocked by 2-aminoethoxy diphenylborate (2-APB), suggesting that they were mediated by IP_3-induced Ca^{2+} release from intracellular stores. In contrast, 2-APB had no effect on Ca^{2+} transients evoked by releasing stored Ca^{2+} with caffeine, suggesting that it had little effect on store Ca^{2+} content. Phenylephrine depressed L-type current by around 30%. This effect was removed by blocking with 2-APB. Notably, phenylephrine failed to enhance the current, even in the presence of 2-APB. Furthermore, the phorbol ester, phorbol 12-myristate 13-acetate, had no effect on L-type current.

Conclusion. Phenylephrine effects on the corpus cavernosum are mediated by modulation of the spontaneous pacemaker mechanism, rather than by direct stimulation of L-type channels. **Doyle C, Sergeant GP, Hollywood MA, McHale NG, and Thornbury KD. Effects of phenylephrine on spontaneous activity and L-type Ca^{2+} current in isolated corpus cavernosum myocytes. J Sex Med 2012;9:2795–2805.**

Key Words. Corpus Cavernosum; Calcium Signaling; Phenylephrine; Chloride Currents; Calcium Current; Detumescence

Introduction

Penile flaccidity is maintained by tonic contraction of the smooth muscle of the corpus cavernosum and penile arteries, thus limiting filling of the corporeal sinuses with blood, hence preventing erection. Conversely, erection is achieved when the smooth muscle relaxes and the sinuses fill with blood, creating turgidity within the enclosed space defined by a surrounding fibrous capsule, the tunica albuginea. It follows that an improved understanding of the process of erection will be

gained by elucidating how smooth muscle tone in the penis is generated and released. Two main factors appear to contribute to tone: (i) a spontaneous myogenic mechanism and (ii) excitatory agonists, the main example of which is neurally released norepinephrine, acting on α_1-adrenoceptors [1,2]. Erection, therefore, occurs by a decrease in sympathetic nerve activity accompanied by neural inhibition of the remaining spontaneous tone, mainly by the action of nitric oxide (NO) released from postganglionic parasympathetic nerves [2,3].

Until recently, it was assumed that myogenic tone in the corpus cavernosum depended on constantly elevated Ca^{2+} concentrations within the smooth muscle cells, mediated by constant Ca^{2+} influx via L-type Ca^{2+} channels [3,4]. In such a scenario, it was proposed that α_1-adrenoceptors enhanced contraction by upregulating the L-type Ca^{2+} channels [4]. Although this idea has not been tested directly, it appears to gain support from studies where contractions induced by α-adrenoceptor agonists were reduced by L-type Ca^{2+} channel antagonists [5–7].

We have recently shown that corpus cavernosum myocytes generated spontaneous Ca^{2+} waves that were capable of traveling most of the length of the cell [8]. In doing so, these waves activated Ca^{2+}-activated Cl^- currents, which caused electrical "slow waves," or depolarizations. The depolarizations, in turn, were enhanced by activation of L-type Ca^{2+} current that resulted in Ca^{2+} influx and activation of more Cl^- current [7]. An obvious idea to test in a "pacemaker"-driven system is that both excitatory and inhibitory agonists exert their effects, at least in part, by modulating the spontaneous mechanism. Previously, we have shown that NO and cyclic guanosine monophosphate abolished both spontaneous Cl^- currents and Ca^{2+} waves in isolated rabbit corpus cavernosum myocytes [8,9]; however, modulation of the mechanism by excitatory agonists has not been examined. The aims of the present study were, therefore, to examine the effects of phenylephrine (PE), a specific α_1-adrenoceptor agonist, on (i) spontaneous Cl^- currents and spontaneous depolarizations, (ii) intracellular Ca^{2+} waves, and (iii) L-type Ca^{2+} current in isolated rabbit corpus cavernosum myocytes.

Methods

Cell Isolation

All procedures were carried out in accordance with European Union legislation and ethical standards and approved by Dundalk Institute of Technology Animal Care and Use Committee. Male New Zealand White rabbits (16–20 weeks old, 25 animals) were euthanized with intravenous pentobarbitone, and the penis removed. The tunica albuginea was opened bilaterally to expose both corpora cavernosa and these were carefully removed, placed in Ca^{2+}-free Hanks solution and cut into 1-mm^3 pieces. The pieces were then incubated in an enzyme medium containing (per 5 mL of Hanks Ca^{2+}-free solution): collagenase 15 mg (type Ia), protease 1 mg (type XXIV), bovine serum albumin 10 mg, and trypsin inhibitor 10 mg for 5–10 minutes at 37°C. All of these chemicals were supplied by Sigma-Aldrich (Arklow, Ireland). The tissue pieces were then placed in Ca^{2+}-free Hanks solution and stirred for a further 5 to 10 minutes to release cells. The cell suspension was stored at 4°C for use within 8 hours.

Patch Clamp Recordings

Patch clamp recordings were made using the amphotericin B perforated patch method, where electrical access between the pipette and cell interior is achieved by inclusion of the pore-forming compound amphotericin B (600 μg/mL) in the pipette solution [10]. Voltage clamp commands were delivered via an Axopatch 1D patch clamp amplifier (Molecular Devices, Sunnyvale, CA, USA) connected to a Digidata 1322A AD/DA converter (Molecular Devices) interfaced to a computer running pClamp software (Molecular Devices). Drugs were delivered via a pipette (tip diameter 200 μm) placed close to the cell. All experiments were carried out at 35–37°C.

Calcium Imaging

Intracellular Ca^{2+} was imaged as described previously [7,8]. Cells were plated and incubated in 0.4 μM fluo-4AM (Invitrogen Corporation, Carlsbad, CA, USA) in "Hanks Ca^{2+}-free solution" (see below) but with added 100 μM Ca^{2+} for 6–8 minutes at 20°C. They were then continuously perfused in physiological saline (see below) containing 1.8 mM Ca^{2+} and imaged using an iXon 887 EMCCD camera (Andor Technology, Belfast, UK) coupled to a CSU22 spinning disk confocal head (Yokogawa, Tokyo, Japan) and Nikon TE200-U inverted microscope with a x40 oil immersion lens (Nikon Corporation, Tokyo, Japan). A krypton–argon laser (Melles Griot, Cambridge, UK) excited the fluo-4 at 488 nm, and emitted light was detected at wavelengths >510 nm. Images were acquired at 10 frames per second and analyzed