Role of Chloride Channels in the Regulation of Corpus Cavernosum Tone: A Potential Therapeutic Target for Erectile Dysfunction

Lau Lang Chu, BSc Hons, MSc, and P. Ganesan Adaikan, PhD, DSc, ACS

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

DOI: 10.1111/j.1743-6109.2007.00728.x

ABSTRACT

Introduction. Recent electrophysiological recordings have identified the existence of outward, excitatory chloride (Cl^-) currents in rat, rabbit, and human corpus cavernosum (CC) muscle cells.

Aim. The aim of this study was to investigate the physiological role of Cl^- currents in the maintenance of cavernosal muscle tone in isolated rabbit CC tissues.

Methods. CC strips ($1 \times 1 \times 5$ mm) were suspended in tissue bath chambers for isometric tension experiments. Spontaneous cavernosal tone and contractions elicited by field stimulation or administration of established smooth muscle constrictors were examined in the presence of chloride channel (ClC) blockers, niflumic acid (NFA), and anthracene-9-carboxylic acid (A9C).

Main Outcome Measure. Both spontaneous myogenic activity and contractile responses to field stimulation, norepinephrine, histamine, and endothelin-1 were reduced by ClC blockers.

Results. In CC strips exhibiting intrinsic myogenic tone, NFA (30 and 100 μM) and A9C (1 mM) caused a relaxation of the tone. In addition, spontaneous contractile activity in CC was abolished in the presence of either ClC blocker. In CC strips precontracted with norepinephrine, histamine, and endothelin-1, both ClC blockers significantly reversed the tone. The ability of NFA and A9C to reverse norepinephrine-induced tone was unaffected by N^{ω}-nitro-L-arginine, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one, and *cis*-N-[2-phenylcyclopentyl]-azacyclotridec-1-en-2-amine. In addition, neither indomethacin nor tetraethylammonium affected the relaxant response of NFA. NFA and A9C inhibited the neurogenic and norepinephrine-induced contractions in a concentration-dependent manner. While NFA exerted persistent inhibition on neurogenic contraction, inhibition of neurogenic contractions by A9C was readily reversible. On K^+-depolarized CC, NFA induced a concentration-related relaxation, whereas A9C was inert, suggesting an additional mechanism of NFA on voltage-gated calcium channels.

Conclusions. These results underline the importance of Cl^- currents as a mechanism in the maintenance of cavernosal tone produced by adrenergic and various endogenous constrictors. Thus, the modulation of Cl^- current, as an attractive and effective approach to regulate penile erection, and specific ClC blockers, as potential erectogenic agents, merits further research. **Lau LC, and Adaikan PG. Role of chloride channels in the regulation of corpus cavernosum tone: A potential therapeutic target for erectile dysfunction. J Sex Med 2008;5:813–821.**

Key Words. Calcium-Activated Chloride Channels; Corpus Cavernosum; Niflumic Acid; Anthracene-9-carboxylic Acid; Erectile Physiology; Drug Therapy

Introduction

Fundamental to penile erection is a complex neurovascular event dependent on the balance between the endogenous contractile and relaxatory mechanisms in the cavernosum tissue. Recent pharmacological manipulation of cavernosal tone using phosphodiesterase type 5 (PDE5) inhibitors has led to remarkable success in the management of erectile dysfunction (ED). However, the efficacy of PDE5 inhibitors is primarily dependent on the integrity of nitric oxide/cyclic guanosine

monophosphate (NO/cGMP) pathway [1]. An alternative approach by disrupting the tonic contractions responsible for keeping the penis in a flaccid state is expected to facilitate cavernosal relaxation. Agents that are known to induce cavernosum muscle relaxation through blocking contractile pathways include α-adrenoceptor antagonists, endothelin receptor antagonist, rho-kinase inhibitor, and calcium channel blockers [2].

Recent studies in vascular, e.g., portal vein, mesenteric vein, ear artery, and aorta [3–6], and nonvascular smooth muscles, e.g., fundus [7], have implicated an important role for Ca^{2+}-activated Cl^- channels in mediating agonist-induced contractions. These channels also participate in spontaneous contractions of smooth muscle in lower esophageal sphincter and myometrium [8,9]. In addition, Ca^{2+}-activated Cl^- channels, which are expressed in many cell types, are involved in signal transduction for diverse physiological functions such as membrane excitability, osmotic balance, transepithelial chloride movements, or fluid secretion [10]. With regard to penile erection, the role of Ca^{2+}-activated Cl^- channels is relatively obscure until Karkanis and coworkers [11] recently provided some electrophysiological and functional evidence of Ca^{2+}-activated Cl^- channels in human and rat corpus cavernosum (CC).

As excitatory mechanisms contribute to the tonic contraction of the CC in the non-erect state, Ca^{2+}-activated Cl^- channels are potential therapeutic target for erectile disorders. In view of a paucity of pharmacological data on chloride channels (ClCs) in penile erection, the present investigation is undertaken to provide further insight on the role of Ca^{2+}-activated Cl^- channels in regulating CC smooth muscle tone, and to explore the erectogenic potential of two ClC blockers, niflumic acid (NFA) and anthracene-9-carboxylic acid (A9C) on rabbit erectile tissue.

Materials and Methods

Animals

Young adult male New Zealand White rabbits (Harlan, Horst, The Netherlands) (N = 18) weighing 3–4 kg were housed in the animal holding unit of the Yong Loo Lin School of Medicine, National University of Singapore. The animals were maintained under a constant 12-hour light and dark cycle and an environmental temperature of 21–23°C. Food and fresh water were available ad libitum. This study was conducted in accordance with the guidelines for animal experiments and principles for the care and use of animals in research and teaching established by the National University of Singapore.

Rabbit CC Tissue

The rabbits were sacrificed by euthanasia with sodium pentobarbital injection (100 mg/kg) through the ear vein. The entire penis was excised and placed in chilled Tyrode's solution of the following composition (mmol/L): NaCl 137, $NaHCO_3$ 11.9, $CaCl_2$ 1.8, KCl 2.7, $MgSO_4$ 1.1, NaH_2PO_4 0.42, and glucose 5.6.

Each CC was carefully cleared of adherent adipose and muscular tissues, tunica albuginea, and was dissected into three strips. A total of 108 strips were obtained for this study. The CC strips ($1 \times 1 \times 5$ mm) were mounted under 1-g resting tension in 10-mL organ baths containing Tyrode's solution bubbled with 95% O_2 and 5% CO_2, and were maintained at 37°C. An equilibration period of 1 hour was applied to all tissues during which the tissues were washed with fresh Tyrode's solution and the baseline was readjusted to 1g tension.

Changes in tissue responses were measured using isometric force transducers (Ugo Basile, Comerio Italy) connected to PowerLab 4SP electronic data acquisition system running chart software version 5.0 (ADInstruments, Bella Vista, Australia) on a Dell computer.

Effect of ClC Blockers on Spontaneous Contractile Activity or Tone Generated by Norepinephrine, Histamine, Endothelin-1, or K^+

In unstimulated cavernosal strips, ClC blockers (NFA or A9C) were administered in increasing concentrations, and changes in spontaneous tone and contractions were monitored.

CC tissue was precontracted with submaximal concentrations of norepinephrine (10 μmol/L), histamine (10 μmol/L), endothelin-1 (100 nmol/L), or KCl (120 mmol/L). After having obtained a stable plateau of contraction with the contractile agent, NFA or A9C was administered in increasing concentrations. Each CC strip was used for only one exposure to a ClC blocker to eliminate the possibility of carried-over effect. Because dimethyl sulfoxide (DMSO) was used as a diluent for stock solutions of NFA and A9C, the effects of this solvent at equivalent concentrations were evaluated in parallel experiments as time-matched controls. The relaxations were expressed as a percentage decrease in tension of the contraction induced by contractile agonist.