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Enhanced Electrical Field Stimulated Nitrergic and Purinergic Vasoreactivity in Distal vs Proximal Internal Pudendal Arteries

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ABSTRACT

Background: The internal pudendal arteries (IPAs) supply blood to the penis and are highly susceptible to vascular remodeling in rodent models of diabetes, hypertension, aging, and chronic kidney disease, thus contributing to erectile dysfunction. Interestingly, vascular remodeling primarily occurs in the distal and not in the proximal IPA, suggesting distinct local physiologic signaling differences within the IPA.

Aim: To examine the role of purinergic signaling and neurotransmitter release by electrical field stimulation (EFS) in the regulation of proximal and distal IPA vascular tone.

Methods: Proximal and distal IPAs were mounted in wire myographs and vascular responses to phenylephrine, acetylcholine, and 2-(N,N-diethylamino)-diazenolate-2-oxide, diethyl-ammonium salt (DEA NONOate) were measured. EFS-mediated contraction and non-adrenergic non-cholinergic (NANC) relaxation were evaluated in the absence and presence of a nitric oxide synthase antagonist. Purinergic agonist and NANC relaxation responses were assessed in the presence and absence of P2X1 and P2Y1 antagonists. Protein expression of P2X1 and P2Y1 receptors was measured by western blot.

Main Outcome Measures: Proximal and distal IPA contraction and relaxation were measured during increasing agonist administration and EFS in the presence and absence of antagonists.

Results: Proximal and distal IPA concentration response curves to phenylephrine, acetylcholine, and DEA NONOate did no differ. Interestingly, distal IPA exhibited greater EFS-mediated contraction and NANC relaxation compared with proximal IPA. Nitric oxide synthase inhibition completely inhibited distal IPA NANC relaxation but did not affect proximal IPA relaxation. P2X1 or P2Y1 receptor antagonism during NANC relaxation increased distal IPA relaxation but decreased proximal IPA relaxation. Combined P2X1 and P2Y1 receptor antagonism had no effect on proximal IPA relaxation but significantly increased distal IPA NANC relaxation.

Clinical Translation: Understanding neurovascular regulation of IPA vascular tone through nitrergic and purinergic mechanisms could yield new therapeutic targets to improve IPA blood flow and treat vasculogenic erectile dysfunction.

Strengths and Limitations: This study is the first to illustrate the differences in mechanisms responsible for regulating vascular tone in the proximal and distal IPAs. All presented findings are currently limited to ex vivo vascular function.

Conclusion: The regulation of vascular tone differs regionally in the IPA. The distal IPA is controlled through neurotransmitter-mediated NO-dependent mechanisms and increased sensitivity to purinergic P2X1 and P2Y1 receptor inhibition. **Odom MR, Pak ES, Brown DA, Hannan JL. Enhanced Electrical Field Stimulated Nitrergic and Purinergic Vasoreactivity in Distal vs Proximal Internal Pudendal Arteries. J Sex Med 2017;14:1285–1296.**

Key Words: Erectile Dysfunction; Internal Pudendal Artery; Electric Filed Stimulation; Vascular Smooth Muscle; Purinergic Signaling; Neurotransmitter Release

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INTRODUCTION

Erectile function relies on complex signaling pathways that result in peripheral neurotransmitter-mediated regulation of penile vascular tone. Disruption of at least 1 of these neurovascular signaling mechanisms can result in erectile dysfunction (ED), which is prevalent in more than 18% of men older than 20 years in the United States.[1] ED is linked to aging, diabetes, hypertension, and cardiovascular disease and is often accompanied by symptoms of arteriosclerosis, fibrosis, and peripheral neuropathy, which impede blood flow to the penis.[1,2] Furthermore, ED in otherwise asymptomatic men is predictive of future cardiovascular disease, particularly in young men.[3,4]

A common goal of ED therapy is to increase blood flow to the penis. The internal pudendal arteries (IPAs) are the primary arteries supplying blood to the penis and contribute to 70% of the total resistance in the penile vasculature.[5] In humans and rats, the IPA originates from the internal iliac artery, runs along the lateral wall of the pelvis, and terminates by branching into the dorsal and deep arteries of the penis.[6] We and others have defined the proximal IPA as the segment from the internal iliac to the gluteal artery branch and the distal portion as from the gluteal artery to the bifurcation of the dorsal and deep penile arteries.[6,7] Pathologic vascular remodeling in the distal IPA has been observed in rat models of aging, hypertension, and chronic kidney disease, which also demonstrate ED.[6–8] Maio et al[7] found extensive arterial wall calcification in the distal IPA from rats with chronic kidney disease that was absent in the proximal IPA. The cause of the disproportionate pathologic remodeling between the proximal and distal segments of the IPA is unknown. Distal IPAs have been shown to have a thicker smooth muscle layer and a smaller lumen diameter compared with proximal IPAs in rats.[7] To our knowledge, the differences in vasoreactivity between the proximal and distal IPAs have not been examined. Potential physiologic differences in the signaling pathways of proximal and distal IPAs could account for the structural variations observed in pathologic conditions.

Vascular tone can be regulated by adrenergic, cholinergic, and non-adrenergic non-cholinergic (NANC) nerve terminals. Nitric oxide (NO) release through NANC nerve terminals is responsible for the initiation of penile vasodilation during an erection.[9] Traditionally, NANC vasodilation is elicited by using electric field stimulation (EFS) in tissue incubated with antagonists of adrenergic neurotransmitter release and muscarinic receptors.[10,11] However, the role of other neurotransmitters such as vasoactive intestinal peptide, calcitonin gene-related peptide, neuropeptide Y, and purinergic mediators (adenosine 5′-diphosphate sodium salt [ADP] and adenosine triphosphate [ATP]) that also can be released from presynaptic nerve terminals are not accounted for.

ATP is a co-neurotransmitter simultaneously released from adrenergic, cholinergic, and NANC nerve terminals with norepinephrine, acetylcholine, and NO that can mediate contractile and vasodilatory responses.[12] Once released, ATP can be further degraded into ADP, cyclic adenosine monophosphate, and adenosine through ecto-nuclease activity.[13] ATP binds purinergic P2X1 and P2Y1 receptors and ADP has a higher affinity for P2Y1 receptors.[12] Stimulation of endothelial P2Y receptors relaxes the human corpus cavernosum,[14] and stimulation of P2X1 receptors in cavernous smooth muscle inhibits erections in diabetic rats.[15] The role of purines in regulating the vascular tone of the IPA has not been investigated. This study characterizes the adrenergic, NO-dependent, NO-independent, and purinergic regulation of vascular tone within the proximal and distal segments of the IPA.

METHODS

Animals

Adult (3- to 4-month-old) male Sprague-Dawley rats (N = 36; 375–450 g; Charles River Laboratories, Morrisville, NC, USA) were housed in pairs and provided with standard rodent chow and water ad libitum in a temperature-controlled room (22 ± 2°C) with a 12-hour light-dark cycle. Animal experiments were authorized by the East Carolina University animal care and use committee under guidelines set forth by the National Institutes of Health (Guide for the Care and Use of Laboratory Animals, 8th edition) and the American Veterinary Medical Association. Animals were separated into 2 groups and used for myograph (n = 28) and western blot (n = 8) experiments.

Sources of Drugs and Reagents

The following compounds were used for myograph studies and purchased from Sigma-Aldrich (St Louis, MO, USA): (R)-(−)-phenylephrine hydrochloride (PE), acetylcholine chloride (ACh), ADP, potassium phosphate monobasic, potassium chloride, D-(+)-glucose, magnesium sulfate heptahydrate, sodium chloride, calcium chloride dehydrate, α,β-methylene adenosine 5′-triphosphate (α,β-MetATP), guanethidine, and N_ω-nitro-L-arginine methyl ester hydrochloride (L-NAME). 2-(N,N-diethylamino)-diazenolate-2-oxide, diethyl-ammonium salt (DEA NONOate) and NF449 were purchased from Cayman Chemicals (Ann Arbor, MI, USA), and MRS2500, 2-methylthioadenosine diphosphate trisodium salt (2-MeSADP) and tetrodotoxin citrate were purchased from Tocris (Bristol, UK). All compounds were dissolved in distilled water with the exception of atropine and DEA NONOate, which were dissolved in 100% ethanol and further diluted using distilled water.

Vascular Reactivity Studies

Rats were anesthetized by intraperitoneal injection of ketamine and xylazine (90 and 10 mg/kg) and bilateral IPAs were carefully excised and placed in ice-cold Krebs solution (NaCl 130 mmol/L, KCl 4.7 mmol/L, KH_2PO_4 1.18 mmol/L, $MgSO_4$ 1.18 mmol/L, $NaHCO_3$ 14.9 mmol/L, dextrose 5.6 mmol/L, and $CaCl_2$ 1.56 mmol/L in distilled water). IPAs were classified