ORIGINAL RESEARCH—BASIC SCIENCE

Endogenous Vasoactive Peptides and the Human Vagina—A Molecular Biology and Functional Study

Harrina E. Rahardjo, MD,*† Andreas Brauer, MSc,‡ Hans-Jürgen Mägert, PhD,‡ Markus Meyer, MD, PhD,§ Wolfgang Kauffels, MD,¶ Akmal Taher, MD, PhD,† Djoko Rahardjo, MD, PhD,† Udo Jonas, MD, PhD,* Markus A. Kuczyk, MD, PhD,* and Stefan Ückert, PhD*

*Department of Urology & Urological Oncology, Division of Surgery, Hannover Medical School, Hannover, Germany; †Department of Urology, Cipto Mangunkusumo Hospital, University of Indonesia School of Medicine, Jakarta, Indonesia; ‡Department of Biotechnology, Anhalt University of Applied Sciences, Köthen, Germany; §FOCUS IP AG, Basel, Switzerland; ¶Department of Obstetrics & Gynecology, Klinikum Hildesheim GmbH, Hildesheim, Germany

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ABSTRACT

Introduction. Endogenous peptides, such as vasoactive intestinal polypeptide (VIP), C-type natriuretic peptide (CNP), and bradykinin (BK), have been proposed to play a role in the female sexual arousal response by exerting relaxation of clitoral, labial, and vaginal smooth muscle. While the effects of endogenous peptides on the human male erectile tissue have already been described, only very few studies have been conducted to investigate the peptidergic control of female genital tissues, including the vagina.

Aims. To elucidate the expression of mRNA specifically encoding for peptide receptors in the human vagina and the effects of VIP, CNP, and BK on the tension induced by endothelin-1 (ET-1) of isolated human vaginal wall smooth muscle. The production of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) in response to exposure of the tissue to the peptides was also measured.

Methods. The expression of mRNA encoding for receptor proteins specific for VIP, CNP, and BK were investigated by means of molecular biology (reverse transcriptase polymerase chain reaction [RT-PCR] analysis). Using the organ bath technique, the effects of VIP, CNP, and BK (0.1 nM to 1 μM) on the tension induced by 0.1 μM ET-1 of human vaginal strips were investigated. The tissue was also exposed to three different concentrations of VIP, CNP, and BK (0.01 μM, 0.1 μM, 1 μM) and the production of cAMP and cGMP determined by means of radioimmunoassays.

Main Outcome Measures. Characterize the expression of peptide receptors in the human vagina and measure the relaxation exerted by BK, CNP, and VIP on the contraction induced by ET-1 of isolated human vaginal tissue. In addition, the effects of the peptides on the production of cAMP and cGMP were also elucidated.

Results. RT-PCR analysis revealed the expression of mRNA transcripts encoding for the VIP receptors VIP1R/vasoactive intestinal polypeptide receptor type 1 (VPAC1) and VIP2R/VPAC2, CNP receptors natriuretic peptide receptor type A (NPRA), natriuretic peptide receptor type B (NPRB) and natriuretic peptide receptor type C (NPRC), and BK receptor B2R. The tension induced by ET-1 was reversed by the peptides with the following rank order of efficacy: BK (21.7%) > VIP (20.9%) > CNP (13.3%). The relaxing effects of VIP and BK were paralleled by a 4.8-fold and fivefold increase in cAMP, while the production of cGMP was stimulated 38-fold and 119-fold in the presence of CNP or BK, respectively.

Conclusion. Our results are in support of the hypothesis that endogenous peptides may contribute to the control of human vaginal smooth muscle tone through the involvement of the cyclic nucleotide-dependent pathways. **Rahardjo HE, Brauer A, Mägert H-J, Meyer M, Kauffels W, Taher A, Rahardjo D, Jonas U, Kuczyk MA, and Ückert S. Endogenous vasoactive peptides and the human vagina—A molecular biology and functional study. J Sex Med 2011;8:35–43.**

Key Words. Human Vagina; Vasoactive Peptides; Cyclic Nucleotides; Peptide Receptors

Introduction

It is assumed that the vagina plays a significant role in the perception of coital stimulation leading to sexual arousal and, finally, orgasm. In response to visual and/or sensory stimulation, relaxation of vaginal vascular and nonvascular smooth muscle occurs, resulting in an increase in local blood flow and vaginal lubrication. The luminal diameter of the vagina also increases, thus allowing penetration of the male penis during sexual intercourse. Nevertheless, the mediators and mechanisms contributing to this process are only poorly understood [1]. It has been suggested that the gaseous transmitter molecule nitric oxide (NO) and vasoactive peptides, such as vasoactive intestinal polypeptide (VIP), natriuretic peptides (NPs), and bradykinin (BK), may mediate the physiological responses during sexual activity [2–5]. These peptides act through the binding to specific receptors and, thereby, trigger the production of intracellular cyclic nucleotides, cyclic adenosine monophosphate (cAMP) or cyclic guanosine monophosphate (cGMP), which, in turn, leads to the relaxation of genital vascular and nonvascular smooth muscle. The prominent signaling pathway for VIP is the stimulation of the enzyme adenylyl cyclase (AC) and subsequent increase in the production of cAMP [6]. C-type natriuretic peptide (CNP), a member of the family of NPs, activates the membrane-bound (particulate) guanylyl cyclase (pGC), resulting in the production of cGMP. BK has been shown to simultaneously mediate the release of several endogenous vasodilators, such as NO, CNP, prostaglandin, or VIP, leading to the enhancement of smooth muscle relaxation [7,8]. It has been speculated that the normal female arousal process is dependent on the balance between the activity of vasodilator peptides, such as VIP, CNP, and BK, and vasoconstrictor peptides, such as endothelin-1 (ET-1), angiotensin II, and neuropeptide Y. While the role of endogenous peptides in the control of genital smooth muscle tone has been investigated mainly in human male penile erectile tissue (corpus cavernosum), few studies have been conducted to investigate the peptidergic control of the vagina. Respective studies used mainly vaginal tissue isolated from female rabbits or rats [9–11].

In order to elucidate further the significance of endogenous vasoactive peptides in the control of human vaginal smooth muscle tone, it was the aim of this study to investigate, by means of molecular biology, the expression of receptors and receptor subtypes specific for VIP, NPs, and BK, and to determine the relaxation brought about by VIP, CNP, and BK on the tension induced by ET-1 of isolated human vaginal smooth muscle. In addition, the production of cAMP and cGMP in response to exposure of the tissue to the peptides was also investigated.

Material and Methods

Tissue Source

In accordance with the regulations of the local ethical committees of the Klinikum Hildesheim GmbH and Hannover Medical School, human vaginal tissue (full wall specimens) was obtained from 32 women (24 menopausal, eight perimenopausal) aged 46–88 years (mean age: 65 years) who had undergone colporrhaphy surgery. Macroscopically normal tissue was taken from the distal portion of the anterior and posterior vaginal wall, immediately placed in a chilled (4°C) organ protective solution (Custodiol, Franz Köhler Chemie GmbH, Alsbach, Germany) and then transported to the laboratory. All experiments were performed within 6 hours to 8 hours after excision of the tissue.

Molecular Biology Analysis

The expression of mRNA encoding for receptor proteins specifically binding vasoactive peptides was analyzed by means of the reverse transcriptase polymerase chain reaction (RT-PCR). In brief, reaction mixtures were prepared in sterile test tubes (volume: 0.2 mL) using up to 5 μg total RNA extracted from specimens of the vaginal wall, 1 μL 10 mM dNTP, and 1 μL oligo (dT) 12–18 (0.5 μg/μL) and brought to 10 μL with diethyl pyrocarbonate (DEPC)-treated water. The mixtures were incubated for 5 minutes at 65°C, then placed on ice for at least 1 minute. Nine microliters of a mixture comprising 2 μL 10× reverse transcriptase (RT) buffer, 4 μL 25 mM $MgCl_2$, 2 μL 0.1 M dithiothreitol, and 1 μL RaceOUT recombinant ribonuclease inhibitor (40 units/μL) were added followed by incubation for 2 minutes at 42°C. Fifty units of SuperScript II RT (Invitrogen GmbH, Karlsruhe, Germany) were added to each test tube (except for the negative RT control) followed by incubation for 50 minutes at 42°C. The reaction was terminated by heating for 15 minutes to 70°C and then cooling the samples to 0°C. Two units of RNase H were added to each tube followed by incubation for 20 minutes at 37°C. PCR amplification was performed using