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In vitro functional responses of isolated human vaginal tissue to selective phosphodiesterase inhibitors

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Abstract Only little is known as to the significance of the cyclic nucleotide-mediated signal transduction in the control of the function of human vaginal smooth musculature. Recently, the presence of the phosphodiesterase (PDE) isoenzymes 4 (cAMP-PDE) and 5 (cGMP-PDE) in the human vagina was reported. Thus, it was the aim of the study to elucidate the effects of some PDE inhibitors on the tension induced by endothelin 1 (ET-1), as well as on levels of cGMP and cAMP in isolated human vaginal wall tissue. Using the organ bath technique, the ability of norepinephrine (NE), carbachol, serotonin (5-HT), oxytocin and ET-1 to contract isolated vaginal wall muscle strips was evaluated. In another set-up, the effects of the PDE4 inhibitor rolipram and PDE5 inhibitors sildenafil and vardenafil (1 nM–10 µM) on the tension induced by 0.1 µM ET-1 of human vaginal wall tissue strips were investigated. In order to measure drug effects on tissue levels of cGMP and cAMP, vaginal tissue was exposed to different concentrations (0.1, 1 and 10 µM) of the compounds and the accumulation of cyclic nucleotides was determined. The adenylyl cyclase stimulating agents forskolin and nitric oxide donor sodium nitroprusside (SNP) (0.01, 0.1 and 1 µM) were used as reference compounds. While NE, carbachol and oxytocin failed to contract the

vaginal tissue, ET-1 and, to a certain degree, 5-HT elicited contractile responses of the isolated strip preparations. The tension induced by 0.1 µM ET-1 was dose-dependently reversed by the drugs. The rank order of efficacy was sildenafil > forskolin > rolipram ≥ vardenafil > SNP. R_{max} values ranged from 24% (SNP) to 50% (sildenafil). With sildenafil being the only exception, none of the compounds reached an EC_{50} value. The relaxing effects of the drugs were paralleled by a fourfold to tenfold increase in tissue levels of cGMP and/or cAMP. Our results demonstrate that PDE inhibitors can relax human vaginal tissue and increase levels of cyclic nucleoside monophosphates. The findings with regard to the PDE5 inhibitors may indicate that the NO–cGMP pathway is, to a certain degree, involved in the control of vaginal smooth muscle tone. This might be of significance with regard to the pharmacological treatment of disorders connected with female sexual arousal and the ability to achieve orgasm.

Keywords Functional responses · Vaginal tissue · Phosphodiesterase inhibitors

Introduction

To date, the physiology of the female sexuality and its impairments has received increasing recognition among both basic scientists and clinicians. As a result, our knowledge regarding the mechanisms regulating the normal female sexual response is slowly growing. This slow progress is due to the complexity of the factors and their interactions—neuronal pathways, endocrine and local cellular mediators—involved in the biological control of female sexuality. It is assumed that the vagina plays a significant role in the perception of coital stimulation leading to normal arousal and, finally, orgasm. With visual and sensory sexual stimulation, an increase in genital blood flow and the relaxation of the smooth musculature of the vagina occurs, resulting in an increase in vaginal lubrication and the luminal diameter,

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thus allowing penetration of the male penis during sexual intercourse. Nevertheless, the mediators and mechanisms contributing to this process are only poorly understood [1–3]. Numerous studies over the past 15 years have described the regulatory importance of cyclic nucleoside monophosphates (cNMP), especially the cGMP signal transduction cascade, in the induction of penile erection. In contrast, there are only few details known regarding the significance of the cNMP-mediated transmission in the control of the normal function of the human vagina. cNMP are part of a complex pathway mediating cellular reactions on external stimulation and, therefore, are of great importance for the regulation of tissue function. The intracellular concentration of cNMP is regulated by the ratio between their generation by cAMP- and cGMP-synthesizing cyclase enzymes and degradation by phosphodiesterases (PDEs), a heterogeneous group of hydrolytic enzymes which may show specific distribution in different human tissues. Until now, 11 PDE isoenzymes have been described which are characterized by their kinetic properties, e.g. affinity for cAMP and/or cGMP, and sensitivity towards inhibitors and allosteric modulators of enzyme activity [4, 5]. Due to their central role in cellular signal transduction, PDEs are considered as important pharmacological targets, especially in the human genitourinary tract [6–10]. Recently, the expression of various PDE isoenzymes, including PDE4 (cAMP-PDE) and PDE5 (cGMP-PDE), was shown by means of immunohistochemistry in the human vagina [11, 12]. Since the mere expression of a PDE protein does not necessarily indicate a functional significance in the control of tissue function, it is still uncertain which PDE is the predominant one with regard to the regulation of vaginal smooth muscle tension. Thus, it was the aim of the study to elucidate the functional effects of some selective inhibitors of PDE isoenzymes 4 and 5 in isolated human vaginal tissue.

Material and methods

Tissue source

In accordance with the regulations of the local ethical committee of the Hannover Medical School, fresh human vaginal wall tissue was obtained from eight women (three menopausal, five postmenopausal) aged 48–63 years (mean age 53 years) who had undergone pelvic surgery (exenteration) for gynaecological malignancies. Macroscopically normal, non-tumorous tissue was excised from the mid-to-proximal portion of the vaginal tube. Tissue specimens were immediately placed in a chilled organ protective solution (CUSTODIOL, Dr. Franz Köhler Chemie GmbH, Alsbach, Germany) and transported to the laboratory for further preparation. All experiments were performed within 6 h after tissue excision.

Organ bath studies

After careful removal of the epithelial layer, vaginal wall tissue was cut into square-shaped segments (approximately $0.8 \times 0.4 \times 0.2 \text{ cm}^3$) and the strips were mounted in the chambers of a vertical organ bath system (IOA 5306 Organ bath, Föhr Medical Instruments GmbH, Germany). Bath chambers were filled with 10 ml of a modified Ringer–Krebs solution (pH 7.4) of the following composition: NaCl 120 mM, NaHCO_3 25.6 mM, KCl 4.7 mM, CaCl_2 2.5 mM, NaH_2PO_4 1.2 mM, MgCl_2 1.2 mM, glucose 22 mM and $2\text{Na}^+(\text{Ca}^{2+})\text{EDTA}$ 0.1 mM. The bath solution was maintained at a temperature of 37°C and continuously oxygenated with a mixture of 95% O_2 and 5% CO_2 . A pretension of 1 gr was applied and the tissue was allowed to equilibrate for at least 60 min. Contractile responses of the tissue to increasing concentrations of norepinephrine (NE), carbachol, serotonin (5-HT, 0.1, 1 and $10 \mu\text{M}$), oxytocin and endothelin 1 (ET-1) (0.01, 0.1 and $1 \mu\text{M}$) were investigated using strips at basal tension.

In another set-up, vaginal wall tissue was exposed to $0.1 \mu\text{M}$ of ET-1. Strips not generating a total tension of ≥ 0.25 gr were disregarded. Once a stable tension had been reached, the PDE inhibitors rolipram, sildenafil and vardenafil, as well as the nitric oxide donor SNP and the adenylyl cyclase activating agent forskolin were added to the bath chambers in a cumulative manner (1 nM – $10 \mu\text{M}$). Isometric responses of the tissue were registered using a MacLab data acquisition system (Analog Digital Instruments, Castle Hill, Australia). Each drug was tested using five to seven individual strip preparations. Relaxant responses of the isolated vaginal tissue are expressed as percent (%) relaxation of the maximum contraction induced by $0.1 \mu\text{M}$ ET-1.

Assays for cyclic nucleotides

To measure tissue levels of cNMP, cAMP and cGMP, vaginal tissue strips were immersed in 2 ml reaction vials containing Ringer–Krebs solution and gassed with a mixture of 95% O_2 and 5% CO_2 . The tissue was then exposed to rolipram, sildenafil, vardenafil (0.1 , 1 , $10 \mu\text{M}$) or SNP and forskolin (0.01 , 0.1 , $1 \mu\text{M}$). Strips were incubated for 10 min. At the end of the incubation period, the tissue was immediately frozen in liquid nitrogen. The tissue was homogenized in the frozen state and cyclic nucleotides were extracted using 80% ethanol (v/v). Samples were then centrifuged at $3,000g$ for 10 min at 4°C . The supernatant was removed and lyophilized. Following redissolution, samples were assayed for cAMP and cGMP by means of a radioimmunoassay (IBL GmbH, Hamburg, Germany) according to the manufacturer's manual. Each drug concentration was tested threefold to fivefold and assayed in duplicate. The protein content of the pellets remaining after centrifugation was measured using the

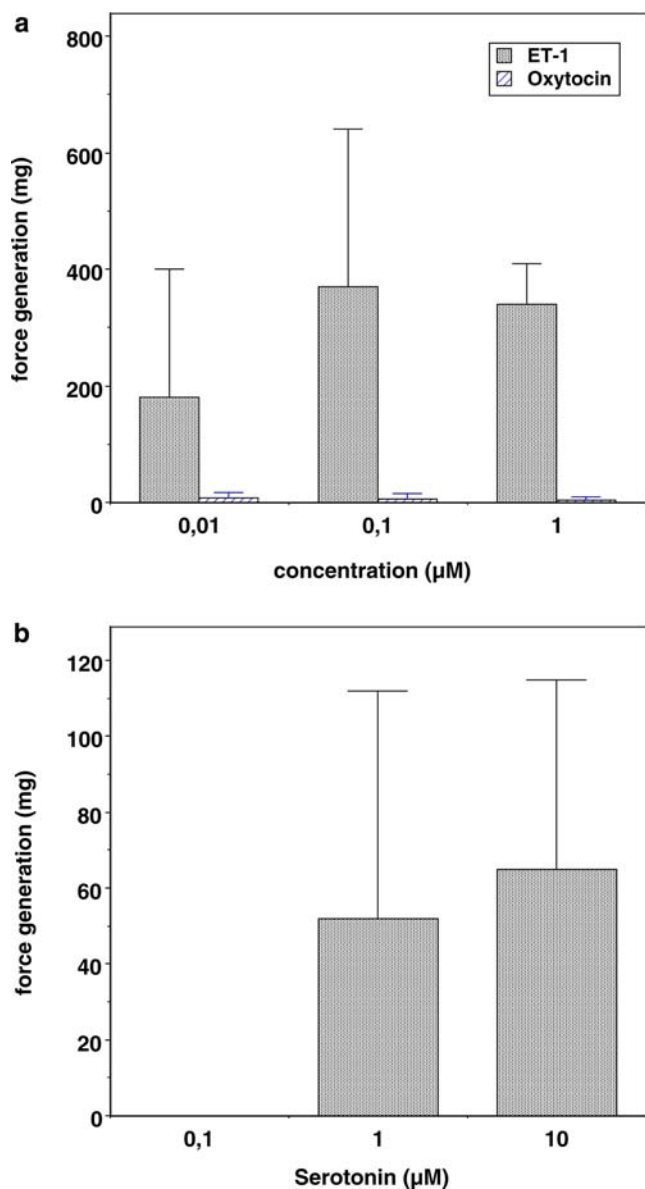


Fig. 1 a, b Contractile responses of isolated human vaginal wall musculature to increasing concentrations of the vasoconstrictory peptides endothelin 1 (*ET-1*) and oxytocin (0.01, 0.1 and 1.0 µM) (a) and the bioactive amine 5-hydroxytryptamine (serotonin 5-HT) (0.1, 1.0 and 10 µM) (b). Each dose–response curve was generated using $n=5-6$ tissue strips originating from at least two different subjects

PIERCE BSA Protein Assay (PIERCE, Rockford, IL, USA).

Statistical analysis

All data are expressed as mean \pm standard deviation (SD) of the mean. The Gosset *t*-test was used to compare the mean values of two or more data cohorts from the organ bath studies or assays for cyclic nucleotides. A probability (*P*) value ≤ 0.05 was considered statistically significant.

Chemicals

SNP and forskolin were obtained from Sigma Chemical Co. (St. Louis, MO, USA), rolipram and 5-HT were purchased from Tocris Cookson Ltd, Avonmouth, UK, the peptides oxytocin and ET-1 were from Bachem AG, Bubendorf, Switzerland. Sildenafil and vardenafil were kindly provided by Bayer HealthCare AG, Wuppertal, Germany. All other laboratory chemicals were either obtained from Merck KgaA (Darmstadt, Germany) or Mallinckrodt-Baker BV (Deventer, The Netherlands).

Results

Organ bath studies

Contractile responses of isolated vaginal wall musculature

In the experiments, ET-1 turned out to be the most effective agent to contract the vaginal wall strips. Cumulative addition of the peptide (0.01, 0.1 and 1 µM) induced reproducible and stable contraction of the isolated tissue amounting to a mean force generation of 180 mg (0.01 µM) and 370 mg (0.1 µM). Increasing the concentration of ET-1 to 1 µM did not result in an enhancement of the contractile response (Fig. 1a). Although 5-HT also induced contraction of the tissue, it was significantly less potent than ET-1. Mean generation of tension induced by 5-HT was 52 and 65 mg only in the presence of 1 and 10 µM of the drug, respectively (Fig. 1b). In contrast, the addition of oxytocin (0.01, 0.1 and 1 µM) did not result in a sufficient generation of tissue tension (Fig. 1a). NE and the muscarinic agonist carbachol also failed to induce contractile responses of the isolated vaginal tissue (data not shown).

Reversion of the ET-1 induced tension by PDE inhibitors, forskolin and SNP

There were only negligible effects of the highest concentration of the vehicles ethanol and DMSO on tension induced by ET-1 of the vaginal wall tissue. The tension was dose-dependently reversed by the drugs. The rank order of efficacy was sildenafil $>$ forskolin $>$ rolipram ($R_{\max} = 38 \pm 22.8\%$) \geq vardenafil ($R_{\max} = 33.3 \pm 19\%$) $>$ SNP. Out of the compounds tested in the study, only sildenafil reached an EC_{50} -value at 10 µM, thus attenuating the tissue tension to a degree of 50% ($R_{\max} = 49.8 \pm 23.2\%$). Neither the NO donor SNP, the adenylyl cyclase stimulating agent forskolin, nor the PDE inhibitors rolipram and vardenafil reached EC_{50} -values. R_{\max} values (reversion of ET-1 induced tension at maximum drug concentration) were determined to be 23.8 ± 19 and $40 \pm 10\%$ for SNP and forskolin, and 38 and 34% for rolipram and vardenafil, respectively. The results are summarized in Fig. 2a, b.

Assays for cAMP and GMP

cGMP levels (control = 0.06 ± 0.04 pmol cGMP/mg protein) were significantly increased threefold to fivefold in the presence of 0.01, 0.1 and 1 μ M of the nitric oxide donor SNP. A dose-dependent stimulation of tissue cGMP production was also noted in response to the PDE5 inhibitors sildenafil and vardenafil, amounting to an elevation of tenfold (0.6 ± 0.3 pmol/mg protein) and eightfold (0.5 ± 0.1 pmol/mg protein), respectively, at a dose of 10 μ M. The most prominent rise in cAMP (tenfold, to 77.7 ± 54 pmol cAMP/mg protein) was reg-

istered in response to 1 μ M of forskolin, whereas 0.1, 1 and 10 μ M of the PDE4 inhibitor rolipram increased cAMP to 16.6 ± 6.0 , 23.8 ± 8.0 and 30.5 ± 8.0 pmol/mg protein, respectively (control = 7.7 ± 4.8 pmol cAMP/mg protein). While the increase in cAMP induced by SNP and sildenafil, as well as the rise in tissue cGMP induced by forskolin and rolipram was not robust nor dose dependent, exposure of the strips to vardenafil (0.1, 1 and 10 μ M) resulted in a linear elevation of cAMP up to 17.0 ± 2.0 pmol/mg protein (2.2-fold). Nevertheless, at concentrations of 0.1 and 1 μ M, this increase was not statistically significant (see Fig. 3a-d).

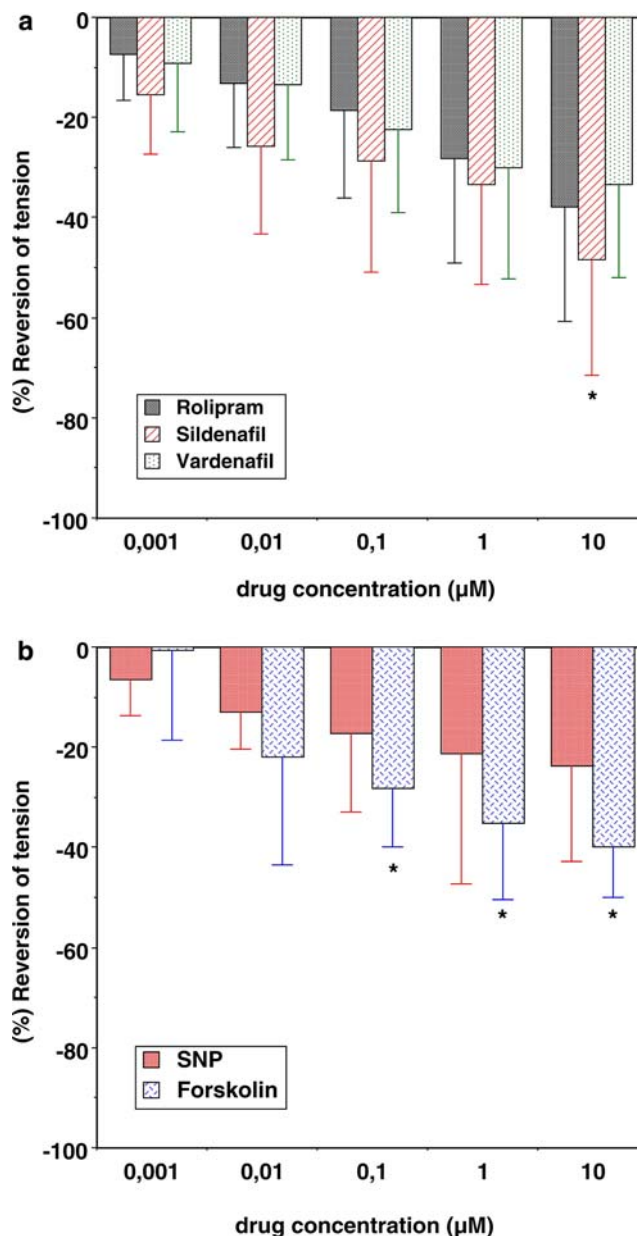


Fig. 2 a, b Reversion of tension induced by 0.1 μ M ET-1 of isolated human vaginal wall tissue strips by increasing concentrations (1 nM–10 μ M) of the PDE4 inhibitor rolipram, the PDE5 inhibitors sildenafil and vardenafil (a), the NO donor drug sodium nitroprusside (SNP) and the adenylyl cyclase activating agent

forskolin (b). $n=5-6$ tissue strips were evaluated per drug concentration and 100% = maximum tension induced by the addition of 0.1 μ M of ET-1. Asterisk indicates statistical significance ($P < 0.05$) when compared to the effect exerted by the lowest drug concentration

Discussion

While research activities have been prevalent during the last two decades on the physiology of the male erectile process, including intracellular signal transduction in the corpus cavernosum, as well as central brain and spinal cord pathways controlling penile erection, the physiology of female sexual response has attracted little attention only. As the basic understanding of local mediators and regulatory factors related to the female sexual physiology offers insight into cellular mechanisms, improved knowledge of the system should advance the

understanding of the functional aspects of female sexual response and the pathophysiology of female sexual disorders. Although the human vagina is intimately connected with the normal progress of the female arousal cycle and sexual eclipse, only very few investigations have so far focused on this organ [13, 14]. The central role of intracellular second messengers cAMP and cGMP in the regulation of various tissues is fairly well established, and because of the theoretical advantage of specific intervention into tissue function by selective inhibitors of PDE isoenzymes, it has been suggested that the concept of PDE inhibition may not only be applicable to the treatment of male erectile

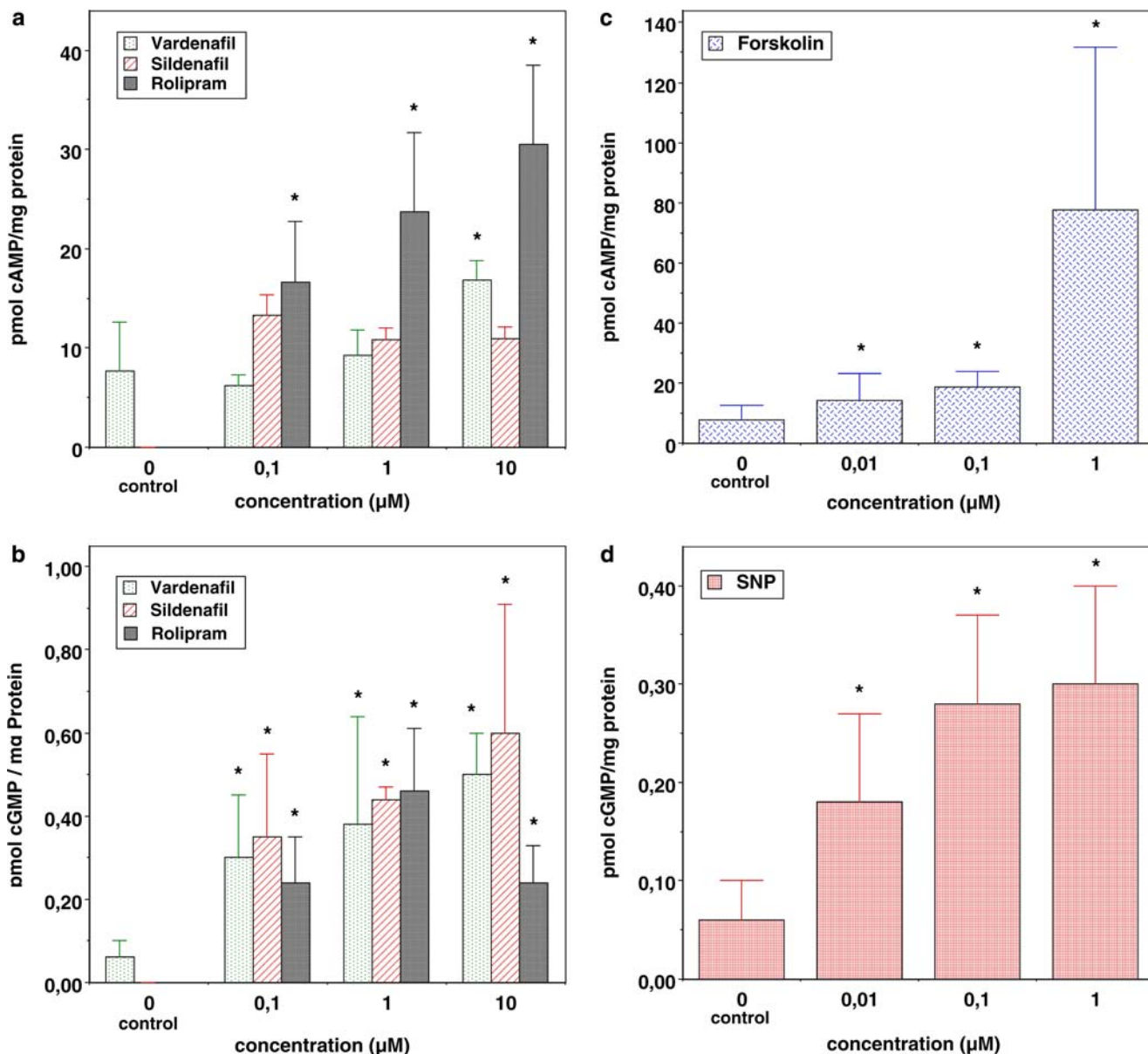


Fig. 3 a–d Assays for cyclic nucleotides: effects of three different concentrations of the PDE4 inhibitor rolipram, the PDE5 inhibitors sildenafil and vardenafil (0.1, 1.0 and 10 μM) (a, b), adenylyl cyclase activating agent forskolin (c) and NO donor drug sodium nitroprusside (SNP) (0.01, 0.1 and 1.0 μM) (d) on tissue

levels of cAMP and cGMP in isolated human vaginal wall musculature. Asterisks indicate significant difference from control ($P < 0.05$ determined by Student's *t*-test). 0 Control (0.06 ± 0.04 pmol cGMP/mg protein and 7.7 ± 4.8 pmol cAMP/mg protein)

dysfunction but also to ureteral colics, urinary incontinence, lower urinary tract symptomatology (LUTS), bladder outlet obstruction (BOO), as well as symptoms of FSD/FSAD [6–10, 15, 16]. Especially with regard to vaginal and clitoral function, it has been speculated as to whether the application of vasoactive drugs, such as PDE inhibitors, known to increase tissue levels of cyclic nucleotides, may facilitate female genital smooth muscle relaxation and enhance vaginal blood flow and secretion, thus resulting in physical dilatation and adequate sexual stimulation [17]. Studies using primary human vaginal cell cultures have suggested that NO and cGMP regulate vaginal smooth muscle contractility and, in addition, the expression of PDE4 and 5 in the human vagina was shown by means of immunohistochemistry [11–14]. Nevertheless, up till now, no studies on the effects of PDE inhibitors on vaginal smooth muscle tone have been carried out. Thus, it was the aim of our study to assess, for the first time, the functional responses of isolated human vaginal wall to the PDE inhibitors rolipram, sildenafil and vardenafil, as well as to the NO donor SNP and adenylyl cyclase activator forskolin.

In initial experiments to evaluate the response of various agents known to contract smooth musculature, the peptide ET-1 turned out to be most effective to induce a stable tension of the vaginal wall smooth muscle strips. Surprisingly, to date, functional studies to characterize transmitter systems and mediators in vaginal smooth muscle have more or less exclusively utilized non-human tissue originating from rat, mouse, rabbit or chicken. The results from these studies suggested that NE, carbachol, prostaglandin F₂ alpha, leukotrienes and vasopressin can contract isolated vaginal tissue [18–20]. While, in our study, no contractile responses of the human tissue preparations to NE and carbachol were registered, the potency of ET-1 to induce long-lasting and stable tension of isolated human vaginal wall, which we observed, has never been described. In the organ bath studies, the PDE5 inhibitor sildenafil proved to be more effective in reversing the tension induced by ET-1 than the other compounds. This relaxing effect was paralleled by a sixfold to tenfold increase in tissue levels of cGMP. In contrast, the stimulation of cGMP and cAMP production, respectively, by SNP (fivefold) and forskolin (tenfold) was not related to a major reversion of vaginal tissue contraction ($R_{\max} < 50\%$). As observed for SNP and forskolin, the relaxation induced by the PDE4 inhibitor rolipram did not exceed 38% of the initial tension plateau although a fourfold increase in cAMP was registered in the isolated vaginal musculature in response to the drug. This apparent discrepancy between the elevation of cyclic nucleotide levels and the mediocre relaxing response of the ET-1 contracted tissue strips may be explained by a possible compartmentalization of cyclic nucleotides within the cell. After tissue penetration, the drugs might demonstrate different distributions within the cell and, thus, elevating cyclic nucleotide levels in different intracellular compart-

ments. Cyclic nucleotides might then act in such a way that pronounced changes in cGMP cause only minor changes in intracellular Ca²⁺ and, subsequently, in smooth muscle tension [21]. When discussing the results of the functional studies, one should also take into consideration that the optimal in vitro action of a PDE inhibitor may require the presence of a threshold concentration of a NO donor or, alternatively, an agent known to stimulate adenylyl cyclase activity in order to trigger the cGMP- or cAMP-cascade and, thus, to ensure that the mechanisms of cyclic nucleotide production and the inhibition of their degradation by PDEs can work synergistically together in the tissue [22, 23].

In conclusion, our results demonstrate that PDE inhibitors can relax human vaginal wall musculature and increase tissue levels of cNMP. The findings with regard to the PDE5 inhibitors may indicate that the NO–cGMP pathway is, to a certain degree, involved in the control of non-vascular vaginal smooth muscle tone. Since the vascular tissue of the human vagina is also considered significant with regard to normal female sexual function, further studies to elucidate the effects of PDE inhibitors and NO donor drugs should also include subepithelial vaginal vessels. Our findings are in support of the hypothesis that cyclic nucleotides and PDE isoenzymes are involved in the control of vaginal function. In the future, in vivo experimental set-ups may reveal as to whether these mechanisms are also involved in temporal events of contraction and relaxation during sexual stimulation.

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