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Phosphodiesterase isoenzymes in the human urethra: A molecular biology and functional study

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MEP 1=EHNA-HCl (erythro-9-(2-hydroxy-3-nonyl)adenine hydrochloride) (PubChem CID: 11957547)
Rolipram (PubChem CID: 5092)
Sildenafil citrate (PubChem CID: 62853)
Vardenafil (PubChem CID: 110634)
Tadalafil (PubChem CID: 110635)

ABSTRACT

Experimental and clinical studies have suggested a role for phosphodiesterase (PDE) isoenzymes in the control of the human lower urinary tract. This study aimed to investigate the expression of PDE isoenzymes and the effects of PDE inhibitors (PDE-Is) in isolated human urethral smooth muscle (USM). The expression of messenger ribonucleic acid (mRNA) specifically encoding for PDE isoenzymes and isoforms (1A, 1B, 1C, 2A, 4A, 4B, 4C, 4D, 5A and 11A) was analyzed by means of reverse transcriptase polymerase chain reaction (RT-PCR). Using a tissue bath technique, the effects of vinpocetine (PDE1-I), erythro-9-(2-hydroxy-3-nonyl)adenine hydrochloride (EHNA-HCl=MEP1) (PDE2-I), rolipram (PDE4-I), sildenafil, vardenafil and tadalafil (PDE5-Is) (0.01–10 μM) on the tension of USM induced by norepinephrine were investigated. The production of cyclic guanosine monophosphate (cyclic GMP) and cyclic adenosine monophosphate (cyclic AMP) was measured by means of radioimmunoassays. RT-PCR analysis revealed the expression of PDE1B, PDE1C, PDE4A, PDE4C, PDE4D, PDE5A and PDE11A. The tension induced by norepinephrine (NE) was reversed by the PDE inhibitors with the following rank order of efficacy: rolipram (mean: −39%) ≥ sildenafil (−35%) > vardenafil (−26%) > tadalafil (−20%) > vinpocetine (−16%) > MEP1 (−2%). The relaxing effects of the drugs were paralleled by an elevation in tissue levels of cyclic AMP and cyclic GMP. Selective inhibitors of PDE4 and PDE5 can antagonize the tension induced by alpha-adrenergic stimulation of USM. PDE inhibition might represent an interesting option to facilitate the relaxation of the human outflow region.

1. Introduction

The normal micturition cycle requires an interaction between the urinary bladder and the outflow region of the lower urinary tract (LUT), this includes the coordinated contraction and relaxation of smooth muscle of both the detrusor and urethra. The control of these functions involves the central and peripheral nervous systems. For example, sympathetic nerves mediate urine storage via the release of noradrenaline, the activation of beta$_3$-adrenoceptors causes relaxation of detrusor smooth muscle, and stimulation of alpha$_{1A}$-adrenergic receptors leads to a contraction of urethral smooth muscle (USM) (De Groat and Yoshimura, 2001). Experimental studies in animals have suggested a potential role of the urethra in maintaining continence and enabling micturition while, in man, the function of USM has been attributed mainly to transient changes in urethral resistance during the active voiding phase (Barnea and Gillon, 2001; van der Werf and Creed, 2002). It has been assumed that the USM is under the control of the nitric oxide (NO)/cyclic GMP pathway and a significance of cyclic GMP specific phosphodiesterase (PDE) isoenzymes – for example, the PDE5 – in this mechanism has been proposed (Andersson, 2001; Hedlund, 2005). To date, the use of selective PDE inhibitors, known to block the degradation of the second messenger molecules cyclic GMP and/or cyclic AMP, offers remarkable opportunities in the treatment of various diseases of the human urogenital tract (Küthe et al., 2000; Oger et al., 2007; Taher et al., 1994; Truss et al., 1996; Ückert et al., 2001, Ückert and Oelke, 2011). However, to date, a few studies only have attempted to examine the role of PDE isoenzymes in the control of USM function.

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Such studies were either limited to urethral tissue isolated from various animals (rabbit, rat, and swine) or – in the case that human tissue specimens were utilized – focused on one PDE isoenzyme only, namely the cyclic GMP PDE5 (Lee et al., 2010; Persson and Andersson, 1994; Tinel et al., 2006; Werkström et al., 2006).

In order to add knowledge to what has been known on the role of PDE enzymes in the human urethra, the present study aimed to investigate by means of molecular biology (reverse transcriptase polymerase chain reaction, RT-PCR) in the human male urethra the expression of cyclic AMP and cyclic GMP PDE isoenzymes PDE1, PDE2, PDE4, PDE5 and PDE11. Using a tissue bath technique, the effects were registered of the PDE inhibitors (PDE In) vinpocetine (PDE1 In), MEP1 (PDE2 In), rolipram (PDE4 In), sildenafil, vardenafil and tadalafil (PDE5 Ins) on the tension induced by the alpha-adrenoceptor agonist norepinephrine of isolated human USM strip preparations. In addition, cyclic GMP and cyclic AMP were also measured following exposure of the urethral tissue to the drugs.

2. Material and methods

2.1. Tissue source

In accordance with the regulations of the local ethical committee of the Hannover Medical School (Hannover, Germany), human urethral tissue was obtained from 21 male subjects (mean age 45 years) who had undergone male-to-female gender reassignment surgery. Macroscopically normal urethral tissue was excised from the corpus spongiosum and transported to the laboratory for further preparation. All experiments were performed immediately after tissue excision.

2.2. Molecular biology analysis

The expression of mRNA specifically encoding for PDE isoenzymes and isoforms 1A, 1B and 1C (Ca^{2+}/calmodulin dependent PDE), 2A (cyclic AMP PDE, stimulated by cyclic GMP), 4A, 4B, 4C and 4D (cyclic AMP PDE), PDE5A (cyclic GMP PDE), and PDE11A (cyclic AMP/cyclic GMP PDE) was analyzed by means of reverse transcriptase polymerase chain reaction (RT-PCR). Using up to 5 μM total RNA extracted from urethral tissue, the protocol was conducted as has been described elsewhere (cycling conditions: initial denaturation for 3 min at 94 °C; 35 cycles of denaturation per 30 s at 94 °C; annealing phase, 30 s at 52–60 °C; extension, 1 min at 72 °C; final extension, 10 min at 72 °C) (Küthe et al., 2000). Primers specifically encoding for β-tubulin were used as positive controls in order to ensure the accuracy of complementary deoxyribonucleic acid (cDNA) first strand synthesis.

2.3. Tissue bath studies

Longitudinal strip preparations (approx. 8 mm × 4 mm × 2 mm) of human penile urethral tissue were mounted to a vertical tissue bath system (IOA 5306, Föhr Medical Instruments GmbH, Seeheim, Germany) and maintained according to standard operation procedures (Ückert et al., 2008). A pre-tension of 1 g was applied and the strips were allowed to equilibrate for 60 min; norepinephrine (NE, 10 μM) was then added. After a stable contraction had been reached, the adenylyl cyclase activator forskolin, NO donor drug sodium nitroprusside (SNP) or the following PDE inhibitors were added to the bath chambers in a cumulative manner (0.01–10 μM): vinpocetine (PDE1-I), MEP1 (PDE2-I), rolipram (PDE4-I), sildenafil, vardenafil and tadalafil (PDE5-Is). The isometric responses of the tissue were amplified and recorded with a MacLab analog-digital converter (AD Instruments, Castle Hill, NSW, Australia). Each drug was tested using 6–8 tissue strips originating from at least 2 different individuals.

2.4. Assays for cyclic nucleotides

To measure levels of cyclic AMP and cyclic GMP following the cumulative administration of the drugs in the tissue bath experiments, the urethral preparations were frozen in liquid nitrogen, homogenized in the frozen state and cyclic nucleotides were extracted using 80% ethanol (v/v). Samples were then centrifuged at 3000*g* for 10 min at 4 °C. The supernatant was removed and lyophilized. After redissolvation, samples were assayed for cyclic AMP and cyclic GMP using specific radioimmunoassays (IBL GmbH, Hamburg, Germany). The protein content of the pellets remaining after centrifugation was measured using the PIERCE BSA Protein Assay (Pierce, Rockford, IL, USA). Each drug was tested using 4 tissue strips originating from 2 different individuals and assayed in duplicate for cyclic nucleotides.

2.5. Statistical analysis

Relaxant responses are expressed as the percentage of the reversion of the maximum contraction induced by 10 μM of NE. The non-specific reversion of tension as a function of time was subtracted from the data readings. All data are given as the mean ± standard deviation of the mean. The Gosset *t*-test was used to compare mean values of the data from the tissue bath studies or assays for cyclic nucleotides. A probability (*P*) value of < 0.05 was considered statistically significant.

2.6. Chemicals

Forskolin and SNP were obtained from Sigma Chemical Co. (St-Louis, USA), norepinephrine-HCl (Arterenol) from Sanofi-Aventis Pharmaceuticals GmbH (Frankfurt am Main, Germany), and MEP1 (EHNA HCl) was purchased from Tocris BioScience Ltd. (Bristol, England, UK). Rolipram was a generous gift from Bayer Pharma AG (Berlin, Germany), tadalafil from Tanabe Seiyaku Pharmaceutical Co. Ltd. (Osaka, Japan), and sildenafil citrate from NicOx SA (Sophia Antipolis, France). All other laboratory chemicals were either obtained from Merck KGaA (Darmstadt, Germany), Lancaster Synthesis (Morecambe, England, UK), or Mallinckrodt-Baker BV (Deventer, The Netherlands).

3. Results

3.1. RT-PCR analysis

RT-PCR analysis revealed the expression of mRNA transcripts specifically encoding for PDE1, PDE4, PDE5 and PDE11. In the agarose gel, clearly visible bands were observed corresponding to the size (in base pairs) of the sequences encoding for PDE1B (510 bp) and 1C (638 bp), PDE4A (692 bp), 4C (650–700 bp) and 4D (683 bp), PDE5A (771 bp), as well as PDE11A (splice variant A1) (500 bp). Either no or only slight bands related to transcripts specific for PDE1A, PDE2A and PDE4B were seen (results not shown). None of the negative controls revealed any visible bands that would have indicated potential contamination of the reaction mixtures subjected to RT-PCR amplification. The results from the molecular biology studies are displayed in Fig. 1(A–D).

3.2. Tissue bath studies

The tonic contraction of the USM induced by 10 μM NE was dose-dependently reversed by the drugs with the following rank