Powerful Relaxation of Phosphodiesterase Type 4 Inhibitor Rolipram in the Pig and Human Bladder Neck

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ABSTRACT

Introduction. Phosphodiesterase type 5 (PDE5) inhibitors act as effective drugs for the treatment of lower urinary tract symptom (LUTS). There is a poor information, however, about the role of the PDE4 inhibitors on the bladder outflow region contractility.

Aim. To investigate PDE4 expression and the relaxation induced by the PDE4 inhibitor rolipram versus that induced by the PDE5 blockers sildenafil and vardenafil, in the pig and human bladder neck.

Methods. Immunohistochemistry for PDE4 expression, myographs for isometric force recordings and fura-2 fluorescence for simultaneous measurements of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and tension for rolipram in bladder neck samples were used.

Main Outcome Measures. PDE4 expression and relaxations to PDE4 and PDE5 inhibitors and simultaneous measurements of $[Ca^{2+}]_i$ and tension.

Results. PDE4 expression was observed widely distributed in the smooth muscle layer of the pig and human bladder neck. On urothelium-denuded phenylephrine (PhE)-precontracted strips of pig and human, rolipram, sildenafil and vardenafil produced concentration-dependent relaxations with the following order of potency: rolipram> > sildenafil>vardenafil. In pig, the adenylyl cyclase activator forskolin potentiated rolipram-elicited relaxation, whereas protein kinase A (PKA) blockade reduced such effect. On potassium-enriched physiological saline solution (KPSS)-precontracted strips, rolipram evoked a lower relaxation than that obtained on PhE-stimulated preparations. Inhibition of large (BK_{Ca}) and intermediate (IK_{Ca}) conductance Ca^{2+}-activated K^+ channels, neuronal voltage-gated Ca^{2+} channels, nitric oxide (NO) and hydrogen sulfide (H_2S) synthases reduced rolipram responses. Rolipram inhibited the contractions induced by PhE without reducing the PhE-evoked $[Ca^{2+}]_i$ increase.

Conclusions. PDE4 is present in the pig and human bladder neck smooth muscle, where rolipram exerts a much more potent relaxation than that elicited by PDE5 inhibitors. In pig, rolipram-induced response is produced through the PKA pathway involving BK_{Ca} and IK_{Ca} channel activation and $[Ca^{2+}]_i$ desensitization-dependent mechanisms, this relaxation also being due to neuronal NO and H2S release. **Ribeiro ASF, Fernandes VS, Martínez-Sáenz A, Martínez P, Barahona MV, Orensanz LM, Blaha I, Serrano-Margüello D, Bustamante S, Carballido J, García-Sacristán A, Prieto D, and Hernández M. Powerful relaxation of phosphodiesterase type 4 inhibitor rolipram in the pig and human bladder neck. J Sex Med 2014;11:930–941.**

Key Words. Phosphodiesterase Inhibitors and Urogenital Function; PDE4 Expression; Rolipram; PDE 4 and 5 Inhibitors; PKA; K^+ Channels; Ca^{2+} Signaling; NO; H_2S; Pig and Human Bladder Neck

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Introduction

Phosphodiesterases (PDEs) are essential components in the cyclic AMP (cAMP)/protein kinase A (PKA) and the cyclic GMP (cGMP)/protein kinase G (PKG) signaling pathways since they can hydrolyze, within the cell, ten times more cAMP and/or cGMP than they are synthesized by their respective cyclases. PDEs inhibition results in large increases in intracellular cAMP and/or cGMP concentrations and subsequent kinase activation, thus enhancing smooth muscle relaxation [1,2]. There are 11 known PDE isoenzymes of which the PDE4, PDE7 and PDE8 families specifically hydrolyze cAMP, whereas that PDE5, PDE6 and PDE9 are cGMP-specific. PDE1, PDE2, PDE3, PDE10 and PDE11 are dual substrate PDEs [1,2].

PDE4 expression and the inhibitory effects elicited by the PDE4 blocker rolipram on the excitatory neurotransmitters have widely been documented in several vascular and visceral tissues. Briefly, PDE4 is present in human umbilical and cavernous arteries smooth muscle and rolipram relaxations have been obtained on 5-hydroxytryptamine (5-HT)-induced precontraction in human umbilical and bovine pulmonary arteries [3–5]. In rat, rolipram increases clitoral and vaginal blood flow induced by clitoral nerve stimulation or prostaglandin E1 [6]. PDE4 has also been described in human prostate smooth muscle [7]. In this structure and in human corpus cavernosum and seminal vesicle, rolipram induced a powerful relaxation of strips prestimulated with noradrenaline [8–10]. At the central nervous system, rolipram has been proposed as potential antipsychotic drug, due to its capacity to increase intracellular cAMP levels and to modify the dopamine D2 receptor binding in brain [11,12].

The cAMP signaling pathway is essential in the regulation of bladder contractility [2,13–15]. cAMP, via PKA activation, regulates smooth muscle function by targeting the activity of various K^+ and Ca^{2+} channels, thus reducing the myocyte excitability and contractility [13,15,16]. The cAMP and cGMP pathways have a major role in detrusor and urethra, respectively, relaxation, and thus PDE4 and PDE5 inhibitors have been proposed for the treatment of overactive bladder and lower urinary tract symptoms (LUTS), respectively [15–29].

The bladder neck is part of the urine bladder outflow region, in which nitric oxide (NO) and non-NO mediators, such as hydrogen sulfide (H_2S), adenosine 5′-triphosphate (ATP), and peptides, as pituitary adenylate cyclase-activating polypeptide 38 (PACAP38), are involved in the inhibitory transmission [30–34]. The knowledge of the mechanisms involved in the control of the bladder neck smooth muscle tone is essential in order to provide therapeutical agents for relaxation of the bladder outlet region during the voiding phase under pathophysiological conditions, such as LUTS [35]. The use of the PDE 5 inhibitors sildenafil, vardenafil and tadalafil improves the symptoms and quality of life in men with LUTS, erectile dysfunction and benign prostatic hyperplasia, possibly due to its effects on cGMP signaling and/or modification of afferent input from bladder, urethra and prostate [36–39]. Poor data exist, however, about the activity of PDE4 inhibitors on bladder outflow region smooth muscle.

Aims

To investigate the PDE4 expression and the relaxation elicited by the PDE4 inhibitor rolipram in the pig and human bladder neck.

Methods

Urinary bladders from adult pigs of either sex were selected from the local slaughterhouse. Human bladders were obtained from three organ donors. Two of them were 53- and 30-year old women which died of a cerebrovascular injury and another one was a 43-year old man who died in a traffic crash. The procedure for obtaining human samples was approved by the Ethics Committee, Hospital Universitario Puerta de Hierro-Majadahonda (Madrid, Spain), with prior consent from the families of the organ donors.

Immunohistochemistry

Pig and human bladder neck segments were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4, for 2 to 4 hours at 4°C, and subsequently placed in 30% sucrose in PB for cryoprotection. The tissue was embedded and frozen in OCT compound (Tissue-Tek®), and stored at –80°C.Transversal sections 5 μm thick were obtained by means of a cryostat and preincubated in 10% normal donkey serum in PB containing 0.3% Triton-X-100, for 2–3 hours. Then, sections were incubated with goat anti-PDE4 (anti-NPP4 C-15, Santa Cruz Biotechnol-

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