Platelet-Derived Growth Factor Regulation of Type-5 Phosphodiesterase in Human and Rat Penile Smooth Muscle Cells

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ABSTRACT

Introduction. Relaxation of cavernous smooth muscle cells (SMCs) is a key component in the control of the erectile mechanism. SMCs can switch their phenotype from a contractile differentiated state to a proliferative and dedifferentiated state in response to a change of local environmental stimuli. Proliferation and contraction are both regulated by the intracellular second messengers cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), which are degraded by phosphodiesterases (PDEs). The most abundant PDE present in corpora cavernosa is the electrolytic cGMP-specific phosphodiesterase type 5 (PDE5).

Aim. We investigated the cellular localization of PDE5 in *in vitro* cultured corpora cavernosa cells and the effect of mitogenic stimulation on PDE5 expression.

Methods. Biochemical ad molecular techniques on cultured SMCs from human and rat penis.

Main Outcome Measures. We studied the ability of the quiescent SMC phenotype vs. the proliferating phenotype in modulation of PDE5 expression.

Results. We demonstrated that PDE5 is localized in the cytoplasm, in the perinuclear area, and in discrete cytoplasmic foci. As previously demonstrated in human myometrial cells, the cytoplasmic foci may correspond to centrosomes. In corpora cavernosa, PDE5 protein levels are strongly regulated by the mitotic activity of the SMCs, as they were increased in quiescent cultures. In contrast, treatment with platelet-derived grow factor (PDGF), one of the most powerful mitogenic factors for SMCs, reduces the expression of PDE5 after 24 hours of treatment.

Conclusion. We found that PDGF treatment downregulates PDE5 expression in proliferating SMCs, suggesting that PDE5 may represent one of the markers of the contractile phenotype of the SMCs of corpora cavernosa. **Carosa E, Castri A, Forcella C, Sebastiani G, Di Sante S, Gravina GL, Ronchi P, Cesarini V, Dolci S, Di Stasi S, Lenzi A, and Jannini EA. Platelet-derived growth factor regulation of type-5 phosphodiesterase in human and rat penile smooth muscle cells. J Sex Med 2014;11:1675–1684.**

Key Words. Phosphodiesterases; Corpora Cavernosa; Type-5 Phosphodiesterase; Cell Proliferation; Platelet-Derived Growth Factor; Cavernosal Smooth Muscle Cells

Introduction

Vascular smooth muscle cells (SMCs) are highly specialized cells in adult animals. Although their principal function is contraction, different stimuli can shift them from the quiescent to the proliferative phenotype. The proliferation of SMCs is critical for the repair of vascular injury, and

its deregulation can result in the development of severe diseases, including atherosclerosis [1], hypertension [1], asthma [2], reproductive disorders [3,4], and cancer [1]. Since phenotypic modulation of the proliferative state of SMCs plays a critical role in the pathogenesis of a variety of cardiovascular diseases, it could have a key role in the pathogenesis of erectile dysfunction, the penis being considered as an extension of the vascular system [1,5].

In the male copulatory organ, the relaxation of vascular SMCs controls erection of the cavernous tissue. Relaxation is chiefly triggered by nitric oxide that activates guanylate cyclase resulting in an enhanced formation of cyclic guanosine monophosphate (cGMP) [6]. The intracellular level of cGMP is tightly controlled both in its rate of synthesis by guanylyl cyclase levels and in its rate of hydrolysis by cyclic nucleotide phosphodiesterases (PDEs) [7,8]. PDEs form a superfamily of enzymes that catalyze the hydrolysis of 3′,5′-cyclic nucleotides to the corresponding nucleotide 5′-monophosphates, which do not activate cyclic nucleotide effector proteins. On the basis of their substrate specificities, kinetics, allosteric regulators, inhibitor sensitivities, and amino acid sequences, at least 11 distinct PDE families have been identified, in total containing more than 50 different PDE enzyme variants, each encoded by several genes [7–10]. Furthermore, each family, and even members within a family, exhibits distinct tissue, cell, and subcellular expression patterns. In corpora cavernosa, the predominant form of PDE is type 5, which is from 10- to 100-fold higher than in other tissues [11]. It has been hypothesized that the overexpression of phosphodiesterase type 5 (PDE5) in corpora cavernosa reflects a physiological condition in which a rapid hydrolysis of the pro-erectile cGMP allows penile SMCs to remain in the contracted state for the majority of the time. However, the molecular mechanism underlying the high expression of PDE5 transcripts in the corpora cavernosa remains to be clarified.

Our aim was to show the cellular localization of the PDE5 in the SMCs of the corpora cavernosa using as model the rat and human penis. Furthermore, we studied the ability of the quiescent phenotype vs. the proliferating phenotype in modulation of PDE5 expression. Hence, the final scope of our experimental design was to clarify if PDE5 is regulated by cell proliferation and if it could be considered a marker of contractile, differentiated phenotype in penile SMCs.

Materials and Methods

Animals

Male Wistar rats were reared in our Institute's facilities, and all the experimental protocols were approved by the Local Ethical Committee in accordance with the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals (DHEW Publication, NIH, 2011) as previously described [12]. The penises from rats at 60 days *post natum* (*dpn*) were carefully excised and separated from the cutis, *os* penis [13] and preputial glands and were used either for cell cultures or frozen in liquid N_2 for RNA and protein extraction or were fixed for immunohistochemistry.

Patients

After protocol approval by the local Clinical Investigation Committee, in accordance with the Declaration of Helsinki, human corpora cavernosa (hCC) were obtained from 10 impotent men (age 72 ± 10.7) at the time of penile prosthesis implantation [14]. After surgery, the corpora cavernosa were immediately used for cell culture preparation or frozen in liquid N_2 for RNA extraction.

SMCs Cultures

Primary cultures of rat and human smooth muscle corpus cavernosum cells were obtained through substantial modification of the methods used by Krall et al. [15]. Briefly, small pieces of corpora cavernosa (approximately 1-mm square) were cut from the penis excised from rats and/or from hCC. Sterile forceps were used to press the fragments onto the bottom of cell culture wells containing Dulbecco's Modified Eagle Medium (D-MEM; Gibco by Life Technologies Ltd., Paisley, UK) + 20% fetal bovine serum (FBS; Sigma-Aldrich Co., Madison, WI, USA), 2 mmol/L glutamine (Gibco), 100 IU/mL penicillin (Gibco), and 100 μg/mL Streptomycin (Gibco). The fragments were incubated undisturbed for 4–6 days at 37°C in fully humidified atmosphere with 5% CO_2. After 4–6 days, the medium was replaced with fresh grown medium. The cultures were incubated undisturbed until 50% confluency was reached, when the remaining tissue fragments were removed and the medium was changed. When the cultures were confluent, the cells were split 1:3 and plated in D-MEM + 10% FBS. This transfer procedure was repeated for subsequent passages. With this method, we obtained a culture of SMCs from the corpora cavernosa of 60 (rCC60) *dpn* rats and