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From Lab to Clinic

Immunohistochemical Distribution of cAMP- and cGMP-Phosphodiesterase (PDE) Isoenzymes in the Human Prostate

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Abstract

Objectives: With the introduction of sildenafil citrate (VIAGRA™), the concept of phosphodiesterase (PDE) inhibition has gained tremendous interest in the field of urology. Cyclic nucleotide second messengers cGMP and cAMP have been assumed to be involved in the control of the normal function of the prostate. The aim of the present study was to evaluate by means of immunohistochemistry the expression and distribution of some cAMP- and cGMP-PDE isoenzymes in the prostate.
Material & Methods: Cryostat sections (10 μM) of formaldehyde-fixated tissue segments excised from the transition zone of human prostates were incubated with primary antibodies directed against the PDE isoenzymes 3, 4, 5, and 11. Then, sections were exposed to either fluorescein isothiocyanate- (FITC) or Texas Red- (TR) labeled secondary antibodies and visualization was commenced by means of laser fluorescence microscopy.
Results: TR-immunofluorescence indicating the presence of PDE4 (cAMP-PDE) was abundantly observed in the fibromuscular stroma as well as in glandular structures of the transition zone. In contrast to the distribution of PDE4, immunoactivity indicating PDE5 (cGMP-PDE) and 11 (dual substrate PDE) was mainly observed in glandular and subglandular areas. No immunostaining for PDE3 (cGMP-inhibited PDE) was detected.
Conclusion: Our results confirm the presence of PDE isoenzymes 4, 5 and 11 in the transition zone of the human prostate and present evidence that these isoenzymes are not evenly distributed. These findings are in support of the hypothesis that there might be a rationale for the use of PDE inhibitors in the pharmacotherapy of BPH and LUTS.

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1. Introduction

The so-called Benign Prostatic Syndrome (BPS), which is characterized by the main clinical items Benign Prostatic Enlargement (BPE), Lower Urinary Tract Symptomatology (LUTS) and Bladder Outlet Obstruction (BOO), represents a major health care problem in most westernized countries [1,2]. It is estimated that approximately 80% of men older than 50 years have moderate to severe symptoms arising from LUTS and that 25% of these will seek medical attention for relief of clinical BPE [3].

The current pharmacological management of LUTS and BPE involves intervention into the hormonal control of prostate growth by using inhibitors of 5-alpha-reductase activity, such as finasteride and dutasteride, to terminate the formation of the active androgen 5-alpha-dihydrotestosterone [4,5]. Alpha$_1$-adrenergic blockers, such as doxazosin, prazosin and terazosin, are also used in order to reduce the sympathetic tone of prostatic smooth muscle cells of the transition zone and the periurethral region [6]. However, nearly 20% of all patients presenting with LUTS/BPE have to undergo prostatic surgery to achieve effective relief of symptoms [7]. Thus, various attempts focus on new therapeutical strategies either to inhibit the proliferation of prostatic stromal and glandular tissue or reverse the tone of prostate smooth musculature. These strategies include the use of drugs to control prostate growth by means of plasma estrogen depletion [8–10], as well as antagonists of the peptide endothelin 1 (ET-1) to induce relaxation of prostate smooth muscle [11].

Experimental studies have demonstrated a fundamental significance of cyclic nucleoside monophosphates cAMP and cGMP (cNMP) in the regulation of human urogenital tract smooth musculature. cNMP are synthesized by the activity of adenylyl- and guanylyl cyclases and are degraded by cyclic nucleotide phosphodiesterases (PDE), a heterogenous group of hydrolytic enzymes. To date, the existence of 11 PDE isoenzyme families is well established and several drugs are known to selectively inhibit PDE isoenzymes [12]. With the introduction of the PDE5 inhibitor sildenafil citrate (VIAGRA^{TM}) as an oral therapy for the treatment of erectile dysfunction, the pharmacological concept of PDE inhibition has gained broad acceptance in the field of urology. More recently, the presence of mRNA transcripts encoding for at least 15 different PDE isoforms (PDE1A, 1B, 1C, PDE2A, PDE4A, 4B, 4C, 4D, PDE5A, PDE7A, PDE8A, PDE9A, PDE10A, PDE11A3, PDE11A4), as well as the hydrolytic activities of PDE isoenzymes 4 and 5 were detected in the human prostate. Moreover, the ability of inhibitors of PDE4 and 5 to reverse the adrenergic tension of prostatic strips isolated from the transition zone was described [13]. To further delineate a potential pharmacological significance of PDEs in the control of the human prostate, it was the aim of the present study to evaluate the presence of PDE isoenzymes 3, 4, 5 and 11 in the transition zone of the organ by means of immunohistochemical methods (immunofluorescence).

2. Material and methods

2.1. Tissue source and handling

In accordance with the regulations of the local ethical committee, human prostate tissue was obtained from 15 male patients (aged 54–76, mean age 65 years) who had undergone radical surgery for localized carcinoma of the prostate or urinary bladder. Macroscopically normal, non-tumorous tissue was excised from the transition zone and immediately placed in an ice-cold solution of 4% formaldehyde in phosphate buffered saline (PBS, pH 7.4).

2.2. Immunohistochemistry

Tissue specimens were immersion-fixated for 4 h in an ice-cold solution of 4% formaldehyde in phosphate buffered saline (PBS, pH 7.4) and rinsed at least three times over 48 h with ice-cold PBS containing 15% sucrose. Specimens were embedded in Tissue-Tec (Miles Laboratories Inc., Elkhart, IN, USA) and then frozen in isopentane at −40 °C to −80 °C. The tissue was sliced with a cryostat to sections of 8–10 μM thickness and thaw-mounted onto glass slides. The sections were incubated for 2 h with a mixture of PBS supplemented with 0.2% Triton X-100 and 0.1% BSA, followed by an incubation for 24–48 h with the primary antibodies (Anti-PDE3, -PDE4, -PDE5 and -PDE11) in a proper dilution (1:250). After rinsing the sections with PBS (3 times for 5 min), either fluorescein isothiocyanate- (FITC) or Texas Red- (TR) labeled secondary antibodies (dilution 1:80 and 1:160, respectively) were applied and allowed to incubate the sections for another 2 h. Following mounting of the tissue sections with phenylenediamine, visualization was commenced using a laser fluorescence microscope (Olympus Corp., Osaka, Japan).

2.3. Antibody source

Antibodies directed against PDEs 3, 4 and 11 were purchased from FabGennix Inc., Shreveport, LA, USA. Anti-PDE5 antibodies were a generous gift from Dr. Kenji Omori, Discovery Research Laboratory, Tanabe Seiyaku Pharmaceutical Co., Ltd. (Osaka, Japan). Texas Red (TR) and Fluorescein (FITC) dye conjugated IgGs were obtained from Jackson Immunoresearch Laboratories (West Grove, PA, USA). All other laboratory chemicals were either purchased from Merck KGaA (Darmstadt, Germany) or Sigma Chemical Co. (St. Louis, MO, USA).

3. Results

3.1. *Immunohistochemistry: Immunolocalization of PDE isoenzymes in the human prostate*

Examination of numerous specimens showed that almost all sections were immunopositive for PDE isoenzymes 4 (cAMP-PDE), 5 (cGMP-PDE), and 11 (dual substrate PDE). TR immunofluorescence revealed that PDE4 was abundantly present in the fibromuscular stroma as well as in glandular structures of the transition zone (Fig. 1A). Small arteries interspersing the fibromuscular stroma presented staining in the adventitia to a certain degree only (Fig. 1B). In contrast to the distribution of PDE4, immunostaining indicating the cGMP-specific PDE5, which is the main target protein of sildenafil, and dual substrate PDE11 (cAMP/cGMP PDE) was mainly observed in glandular structures of the transition zone. While PDE5 staining was found to be most prominent in the entire glandular region, immunofluorescence related to the expression of PDE11 was mainly present in epithelial layers (Fig. 1C and D). While relevant immunoreaction for PDE5 was also detected in stromal parts of the sections, the staining indicating the distribution of PDE11 in the fibromuscular stroma of the prostate was inferior when compared to the activity registered in glandular structures. Neither in glandular nor non-glandular tissue sections a positive FITC-immunostaining for PDE3 (cGMP-inhibited PDE) was detected (Fig. 1E). In order to acertain the degree of staining, tissue sections omitting the primary antibodies were used as a control.

4. Discussion

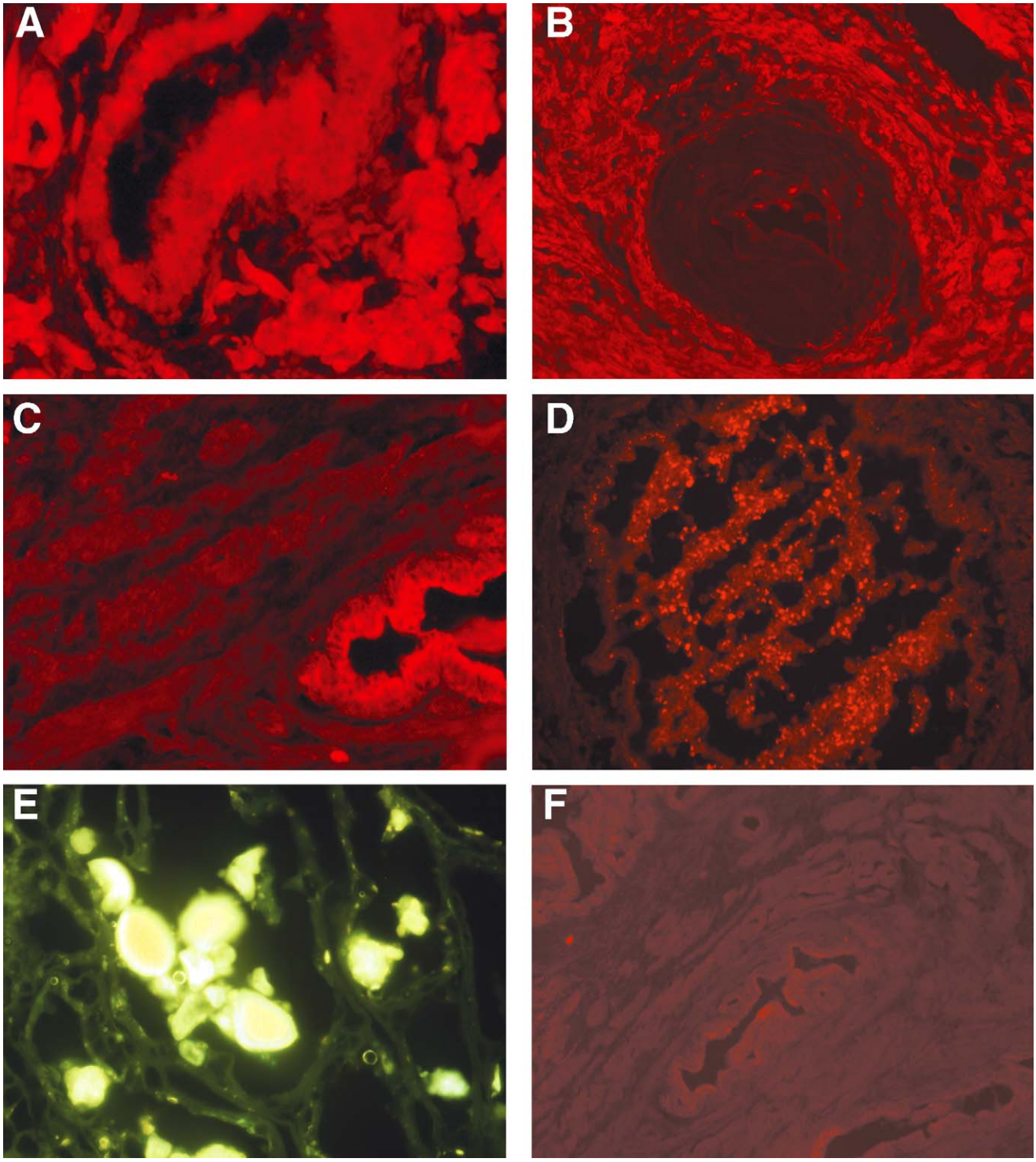
To date, the pharmacological concepts in the therapy of BPS are in a stage of critical evaluation of their efficacy and several new treatment modalities are now being discussed, among which are aromatase inhibitors, polyenantibiotics, potassium channel openers, synthetic ligands of CGRP peptide receptors, and antagonists of the endothelium-derived peptide ET-1 [8–11,14,15].

The central role of intracellular second messengers cAMP and cGMP in the regulation of smooth muscle tone in various tissues including the human prostate is fairly well established [16,17]. The turnover of cAMP and cGMP is regulated by the activity of cyclic nucleotide phosphodiesterase enzymes. Because the distribution and regulatory significance of PDE isoenzymes varies in different tissues, selective inhibitors with the potential to exert at least partially specific effects on the target tissue have been developed [12]. Because of the theoretical advantage of specific intervention into tissue function by inhibitors of PDE isoenzymes, it has been suggested that the concept of selective PDE inhibition may also be applicable in the treatment of various diseases of the male and female genitourinary tract, including ureteral colics, urinary incontinence, and symptoms of female sexual dysfunction [18–20]. To date, the inhibition of cGMP degradation by specific PDE5 inhibitors, such as sildenafil, vardenafil (LEVITRA^{TM}) and tadalafil (CIALIS^{TM}), is a straightforward pharmacological approach to induce penile erection in patients with erectile dysfunction [21].

Using chromatographic techniques, the presence of protein related to the hydrolytic activities of PDE isoenzymes 4 and 5 was demonstrated in cytosolic and microsomal fractions of the transition zone and the periurethral region of the human prostate. RT-PCR analysis revealed the expression of mRNA transcripts encoding for at least 15 different PDE-isoforms including PDE 11A in the organ [13,22]. However, the mere expression of a mRNA does not yield any information on its functional impact since transcripts encoding for distinct PDE isoenzymes are not necessarily translated into an active protein. Moreover, the results from chromatographic studies using anion exchange columns may either mask or pretend the presence of certain PDE isoenzymes due to co-elution of different PDE proteins from the column at a given ionic strength [23].

The results from our present study confirm the occurrence of PDE isoenzymes 4 (cAMP-PDE) and 5 (cGMP-PDE) in the human prostate and reveal that these proteins are not evenly distributed. Although a significant amount of PDE5 immunostaining was seen in the fibromuscular stroma, a majority of the enzyme appeared to be associated with glandular structures. Based on our findings, one can speculate that these isoenzymes are involved in the control of both the glandular and stromal tissue of the transition zone. This involvement may well include the regulation of stromal smooth muscle dynamic activity as well as glandular secretory function and tissue proliferation. Such a hypothesis is strongly supported by previous findings: Organ bath studies

Fig. 1 – (A–F) Laser fluorescence microscopy: Cryostat section of a specimen from the transition zone of the human prostate presenting TR-immunofluorescence specific for PDE4 (cAMP-PDE) in the fibromuscular stroma and in glandular structures (A) (Magnification ×20). (B) displays immunostaining (TR) indicating the presence of cAMP-specific PDE4 in the transition



zone of the human prostate. While abundant staining is observed in the fibromuscular stroma, a small vessel interspersing the stroma appears unlabeled, presenting a weak immunoreaction only in the adventitia (Magnification ×10). (C) Presents dense immunofluorescence (TR) specific for PDE5 (cGMP-specific PDE) in glandular and subglandular structures of the transition zone. Although the immunoreaction observed in the stromal part is different from control (F), it is not as high in degree as the staining observed in the glandular region (Magnification ×20). A cross section of a glandular region of the transition zone surrounded by fibromuscular stroma is characterized by dot-like immunofluorescence (TR) localized in epithelial and subepithelial layers indicating the expression of Dual Substrate PDE11 (cAMP/cGMP-PDE) (D) (Magnification ×20). (E) Presents a detail from a glandular region of the human prostate. The glandular lumen contains prostate stone concrements. While the tissue appears unlabeled, non-specific binding of secondary antibodies to the stone material had occurred (FITC-staining, Magnification ×20).

demonstrated that the norepinephrine-induced tension of human prostate tissue segments isolated from the transition zone was in part reversed by the nitric oxide donor sodium nitroprusside, diterpen forskolin (an enhancer of intracellular cAMP-generation), PDE4 inhibitor rolipram, and PDE5 inhibitors sildenafil and zaprinast [13]. This is well in accordance with earlier findings suggesting that prostatic smooth muscle relaxation is mediated by the elevation of cAMP and cGMP [16,17]. Interestingly, it has been demonstrated that an increase in both intracellular cAMP and cGMP in human prostate cancer cells lines initiates morphologic differentation and inhibits the proliferation and invasive potential of the cells [24,25]. The antiproliferative and pro-apoptotic effects of MY 5445 and the novel cGMP-PDE inhibitor exisulind (sulindac sulfone) and its derivatives CP248 and CP461 have been described and the potential use of these compounds has been suggested as selective apoptotic and anti-neoplastic drugs in the treatment of localized and advanced prostate cancer. Indeed, there are several lines of evidence that in a wide variety of human tumour cell lines, including adenocarcinoma of the prostate, PDE5 is expressed and dominates the hydrolysis of cGMP [26,27].

Therefore, with regard to the multiple physiological roles of cyclic nucleoside monophosphates in the mammalian prostate, the involvement of a cGMP-PDE, such as PDE5, and a cAMP/cGMP-PDE (dual substrate PDE), such as PDE11A, in controlling prostate function seems more than plausible. Our finding that immunoactivity indicating the presence of PDE11A was observed in epithelial and subepithelial glandular structures rather then in stromal parts of the prostatic tissue sections may give way to the speculation that this isoenzyme might rather be of significance in the control of glandular function and tissue proliferation. Nevertheless, on the basis of our observations, it is difficult to predict which PDE isoenzyme – PDE4, 5 or 11 – is the predominant one in the human prostate to modulate intracellular signaling within epithelial and subepithelial glandular tissue of the transition zone. Further work is needed to evaluate this in detail. Pharmacological studies using selective inhibitors for PDE11A will elucidate the particular physiological function of this isoenzyme.

In conclusion, the results from our study confirm the presence of immunoreactive protein related to PDE isoenzymes 4, 5 and 11 in the transition zone of the human prostate and – for the first time – present evidence that these isoenzymes are not evenly distributed in the organ. These findings are in support of the hypothesis that there might be a rationale for the use of PDE inhibitors, which are able to elevate intracellular levels of cyclic nucleotides cAMP and cGMP, in the pharmacotherapy of BPE and LUTS. Such drugs may effectively interfere with physiological mechanisms regulating stromal smooth muscle tone and tissue proliferation [28].

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