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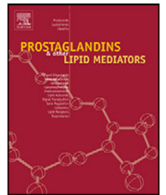

The distribution of the prostaglandin E receptor type 2 (EP2) in the detrusor of the guinea pig

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ABSTRACT

Objective: To explore the distribution of prostaglandin E receptor type 2 (EP2) in the bladder muscle layers and its spatial relationship to cyclo-oxygenase type 1 (COX I).
Materials and methods: Twelve male guinea pigs were killed by cervical dislocation, the bladders removed and fixed in 4% paraformaldehyde in PBS. Frozen sections of 10 μm were cut and stained with antibodies to EP2, COX I and vimentin.
Results: EP2 receptor immunoreactivity is located on the smooth muscle cells as well as on vimentin positive surface muscle and intramuscular interstitial cells. EP2 expression on interstitial cells is highly localized. Discrete regions of intense staining were observed on the interstitial cell processes. COX I is expressed in the muscle interstitial cells and was found to be located on discrete regions of the cell and cell processes. Double staining with EP2 and COX I suggests that the regions of a cell expressing EP2 are different from those expressing COX I.
Conclusions: The presence of COX I, prostaglandin E receptor type 2 (EP2) immune-reactivity in the network of interstitial cells suggests a role of this network in the propagation of signals. Due to a cAMP coupling of the EP2 receptor in many other tissues and a lower dissociation constant of EP2, it is suggested that a rise in PG levels may gradually push the balance from a relaxant EP2 effect towards a contractile effect. Hence, PG could have a modulatory role on the non-voiding bladder contractions by changing the threshold level for excitability of the interstitial cell network.
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1. Introduction

It is now generally accepted that prostaglandin (PG) plays a role in the control of mammalian urinary bladder motility [1,2]. In the bladder, both the lamina propria and the muscle layers can produce prostaglandins [3–6]. Prostaglandin E_2 (PGE_2) is known to be the predominant PG in the urinary bladder of most mammals [7] and the receptors for PGE_2 are classified into four types: EP1, EP2, EP3 and EP4 [8]. PGE_2 is an agonist at EP receptors 1–4, all G-protein coupled, which mediate its physiological effects [9]. PGE_2 is the main PG suggested to be involved in the pathophysiology of detrusor overactivity (DO) and overactive bladder syndrome (OAB) [9,10]. This involvement is based on three observations. Firstly, the ability of PGE_2 infused into the bladder to induce DO in humans and animals. Secondly, the increased PGE_2 production in DO models and thirdly, the high concentrations of PGE_2 that have been detected in the urine of patients with OAB [9]. It has also been shown that upon stretch, PGE_2 is released by the urothelium [11] and that this release is regulated by a complex interaction of signals through ATP and NO [1].

The literature contains substantial data that the wall of the mammalian urinary bladder contains several sub-populations of cells in the interstitial space, called interstitial cells (ICs) after the interstitial cells of Cajal in the gut [12]. These interstitial cells are located in the lamina propria and within the detrusor and have been suggested to form a network called the interstitial cell network [3,13–15]. The expression of prostaglandin E receptor type 1 (EP1) in the guinea pig muscle interstitial cell network has been studied recently [3].

Moreover, it has been shown that the cyclo-oxygenase I (COX I) enzymes are located within specific cell types within the lamina propria of the guinea pig bladder [16]. In addition, COX I is found in the basal layers of the urothelium and associated with the

Abbreviations: PG, prostaglandin; PGE_2, prostaglandin E_2; EP1, prostaglandin E receptor type 1; EP2, prostaglandin E receptor type 2; COX I, cyclo-oxygenase type 1; DO, detrusor overactivity; OAB, overactive bladder syndrome; ICs, interstitial cells; IM-ICs, intramuscle interstitial cells; SU-ICs, surface muscle interstitial cells; PDE, phosphodiesterase.

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distributed network of lamina propria interstitial cells [16]. It has also been suggested that the arrangement of EP1 and COX I might have the potential to facilitate the propagation of signals in the interstitial cell network [3]. Such a signalling system is thought to have a role in coordinating events in the normal bladder [17] and might be defective in bladder pathology [13]. It might also have a function in facilitating the global coordinated changes associated with bladder wall remodelling [3,18].

PG receptors EP1 and EP2 have been shown to be expressed in the ICs in the lamina propria indicating that this part of the bladder wall can respond to PGE_2 [4].

There is hardly any literature about the exact role of the EP2 receptor in the bladder. However, it is known that the normal guinea pig urothelium does express EP2 receptors [4] and that the combined EP1/EP2 receptor antagonist AH 6809 decreases detrusor contraction in isolated human bladder experiments [19]. Unlike EP1 [3], the cellular localization and the expression of EP2 receptor in the muscle layers of the bladder have not been studied before. In the present study, we have used antibodies to EP2 in order to study the distribution of this receptor in the muscle layers of the guinea pig urinary bladder. Our results are discussed in terms of possible physiological mechanisms, which might be occurring in this region of the bladder wall.

2. Materials and methods

Guinea pigs (12 male, weight range 260–300 g) were killed by cervical dislocation. All procedures were carried out in agreement with the guidelines of the Maastricht University Ethical Committee. The tissue workup of the lateral wall of the guinea pig bladders as well as freezing and the cutting of the sections are as described in our previous paper [4].

2.1. Tissue preparation

The bladder, including the proximal urethra, was removed from each animal and placed in ice-cold Krebs solution composed of 121.1 mM NaCl, 1.87 mM KCl, 1.2 mM $CaCl_2$, 1.15 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.17 mM KH_2PO_4 and 11.0 mM glucose bubbled with 5% CO_2 and 95% O_2 (pH 7.4).

Each bladder was divided into a ventral piece and a dorsal piece, while maintained in Krebs solution. Then, the bladder pieces were immersed in ice-cold fixative solution of 4% freshly prepared depolymerized paraformaldehyde for 120 min at 4 °C. Tissues were fixed in 3 steps of 2 overnight and 1 daytime incubation at 4 °C in 0.1 M phosphate buffer with 10%, 20% and 30% sucrose, respectively. On day 3 the tissues were placed in Tissue-Tek® OCT™ compound to form a single block, snap frozen in isopentane and cooled in liquid nitrogen. Cryostat sections (10 μm) were cut such that each section was perpendicular to the urothelial surface. Sections were thawed on chrome alum-gelatin coated slides and processed for immunocytochemistry.

2.2. Characterization of the EP2 antibody

In order to gain insight in the specificity of our antibody, we conducted a pre-absorption test and characterized the antibodies by Western blotting as described in our previous paper [4]. In short, preabsorption was done by overnight incubation of the anti-EP2 antibody (1:100 by Cayman Chemical, Catalogue No. 301740) with or without 10 μg/ml of the peptide against which the antibody was raised. Thereafter the antibody solution or antibody plus peptide solution was applied to the sections.

Bladder homogenate of guinea pig bladders (N = 2) was prepared for Western blotting. Bladders were cut into squares of approximately 1 mm^2 and homogenized using an UltraTurrax homogenizer (Janke & Kunkel, Ika Labortechnik, Staufen, Germany) at 4 °C in 1× radio-immunoprecipitation assay buffer (1% Triton-X-100, 137 mM NaCl, 20 mM Tris–HCl, 2 mM EDTA, 10% glycerol, 1 mM sodium orthovanadate, 10 mM NaF, 1× protease inhibitor cocktails, 1 mM phenylmethylsulphonyl fluoride). Subsequently the homogenates were centrifuged at 13,000 × g for 20 min at 4 °C. The pellet was discarded and the supernatant was stored at −80 °C. Protein measurements were done using bovine albumin serum dilutions with the Biorad protein measurement system (Bio-Rad Laboratories, Inc., Hertfordshire, CA, USA) according to the manufacturer's instructions.

The blot analysis was performed under reducing conditions following standard procedures and using the Odyssey infrared imaging system (Li-cor Biosciences, USA). Primary antibody used for Western blotting was rabbit anti-EP2 antibody (Cayman Chemicals) used in a dilution of 1:100. The staining was also confirmed by a blocking peptide on the Western blot using pre-absorption with anti-EP2 antibody in a dilution of 1:100 (Cayman Catalogue Nos. 301750 and 301740). The secondary antibody used was donkey anti-rabbit IRdye800 (Rockland, 611-732-127).

2.3. Immunohistochemistry

Sections were dried for 20 min at room temperature followed by three washes with Tris–buffered saline (TBS; pH 7.6), and thereafter

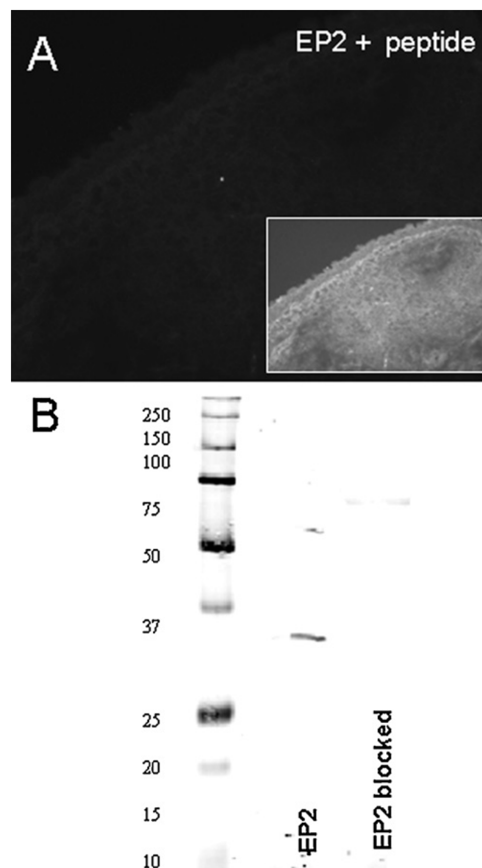

Fig. 1. Characterization of the EP2 antibody. Panel A illustrates the staining observed with the same concentration of antibody as used in all the stainings of other figures, but with pre-incubation with the blocking peptide to which the antibody was raised. (The inset shows an enhanced image showing the section morphology.) Calibration bar: 10 μm. Panel B shows a characterization of the antibodies using Western blotting. Three lanes are shown: the molecular weight calibration bands, the native protein stained with the EP2 antibody and the native protein but exposed to antibody plus appropriate blocking peptide.

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