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Muslim Ali · Maria Angelo-Khattar
Layla Thulesius · Amal Fareed · Olav Thulesius

Urothelial synthesis of prostanoids in the ovine ureter

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Abstract The purpose of this study was to investigate the site of production (urothelium/smooth muscle) and quantitative release of prostanoids in the ureter. Quantitative analysis of prostacyclin (PGI_2) was by its metabolite, 6-keto PGF_{1alpha}, thromboxane (TXB_2) and prostaglandin F_{2alpha}. Synthesis by radiometry and radioimmunoassay was performed in vitro in sheep ureter specimens before and after removal of the inner epithelial layer and after addition of indomethacin. The major prostanoids present in the ureter were PGI_2 and TXB_2; PGI_2 was quantitatively the largest component. Removal of the thin inner epithelial layer (urothelium) reduced mostly PGI_2; addition of arachidonic acid significantly augmented PGI_2 only in ureters with intact urothelium but did not alter TXB_2 levels. The main source of prostanoid synthesis (PGI_2) of the ureter is to be found in the urothelium. The functional role of the prostanoids may be related to coordination of peristalsis.

Key words Prostanoids · PGI_2 · TXB_2 · Ureter · Urothelium · Indomethacin

Introduction

In previous studies we have shown that rhythmic contractile activity of the isolated sheep and human ureter is dependent on the local synthesis of prostanoids, and ureteral peristalsis is blocked by indomethacin and glucocorticoids [4, 5, 10–12]. In another study we identified the major prostanoids of the ureter as prostacyclin (PGI_2), thromboxane B_2 (TBX_2) and prostaglandin F_{2alpha} (PGF_{2alpha}) [3].

Analogous to the situation in blood vessels, it can be expected that normal prostanoid synthesis by the enzyme cyclooxygenase-1 (COX-1) is prominent in the cells lining the lumen (urothelium). The functional consequence of prostanoids with anti-platelet activity probably contributes to the action of urokinase in the prevention of obstructing blood clots in the ureter. The present investigation was designed to investigate the role of the urothelium in the synthesis of prostanoids.

M. Ali · A. Fareed
Department of Biochemistry, Faculty of Science,
Kuwait University, Kuwait

L. Thulesius
Department of Anatomy, Faculty of Medicine,
Kuwait University, Kuwait

M. Angelo-Khattar · O. Thulesius
Department of Pharmacology/Toxicology, Faculty of Medicine,
Kuwait University, Kuwait

O. Thulesius (✉)
Department of Clinical Physiology, University Hospital,
S-581 85 Linkoping, Sweden
Fax +46(13)145949

Materials and methods

Tissue preparation

The specimens were obtained from Australian merino sheep of either sex. Kidneys with attached ureters were harvested early in the morning from the local slaughterhouse and quickly transported, in Krebs' solution on ice, to the laboratory. The ureters were dissected free from adjacent connective tissue and fat and 4-mm-long rings were cut from the proximal part, 2–5 cm from the renal pelvis. A pair of adjacent rings were prepared, one which was not altered and one in which the inner layer of the urothelium had been removed by inserting a forceps and gently rolling it on a cork board in order to remove the inner urothelial layer. The effectiveness of this procedure in removing the urothelium was checked by light microscopy and electron microscopy. Kreb's solution contained: NaCl, 119; KCl, 4.7; $NaHCO_3$, 25; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $CaCl_2$, 2.5 and glucose, 11 mM; pH = 7.4 at 37°C.

Radiometric assay

The whole and rubbed ureter, respectively were cut in small pieces, homogenized in 3 volumes of 0.05 M TRIS-HCl buffer (pH 7.4). The homogenized tissue was centrifuged at 5000 rpm and the supernatant dispensed in 1-ml aliquots and preincubated for 5 min with normal saline or with the addition of indomethacin (25 and 250 nM). The synthesis of prostanoids was initiated by the

addition of ^{14}C arachidonic acid as described by Ali and McDonald [1]. The reaction was allowed to proceed for 30 min at 37°C in a shaking water bath at which time maximum synthesis of prostanoids was observed. The reaction was stopped by the addition of 9.2% formic acid. The total prostanoids synthesized were extracted three times with ethyl acetate (3 volumes) and the pooled extracts were dried completely under a stream of nitrogen and stored in methanol at −70°C until further use. The labelled products were separated from the labelled precursors using minicolumns of silicic acid and thin layer chromatography (TLC) as described previously [2]. Authentic standards of prostaglandins and TBX_2 were detected by spraying with 10% phosphomolybdic acid in ethanol. Quantification of the separated radiolabelled products was achieved by either scanning the TLC plates on a radiochromatogram scanner (Packard Model 7201) or by the elution with 10 ml of methanol and counting of individual peaks using a liquid scintillation counter (Beckman Model LS 5000 TD). Recovery from thin layer plates was approximately 80%. Inhibition of prostanoid synthesis was achieved by the addition of indomethacin (Sigma Chemicals, St Louis Mo.).

Radioimmunoassays

Radioimmunoassay (RIA) of prostanoids was achieved by incubating sheep ureter homogenate with unlabelled arachidonic acid (100 mM concentration). The extraction and separation was carried out as described below. The concentrations of TBX_2 and 6-keto PGF_{1alpha} were measured by a method previously described [2] using RIA kits (Amersham, UK). Appropriate solvent blanks were tested and found to be below the detection limit of the assays.

Analysis

Results are presented as mean ± SE of *n* experiments. Differences between mean values were tested for statistical significance using the two-tailed Student's *t*-test. A significance level below 0.05 was considered significant.

Table 1 Reaction products identified on thin layer chromatograms from extracts of whole ureter homogenate incubated with arachidonate (100 mM)

Prostanoids	Radioactivity in products
6-keto PGF_{1alpha}	64.20 + 4.58 (13)[a]
TXB_2	15.69 + 2.91 (13)
PGF_{2alpha}	7.57 + 1.01 (13)
PGE_2	7.10 + 0.98 (13)
PGD_2	5.37 + 0.86 (13)

[a] Number of independent experiments

Results

Table 1 presents the results of the in vitro synthesis of prostanoids as assessed by TLC from extracts of whole ureter homogenate incubated with ^{14}C arachidonate. As can be seen, by far the most predominant type was 6-keto PGF_{1alpha}, the metabolite of prostacyclin. Under the conditions of the experiment, TXB_2, PGF_{2alpha}, PGE_2, and PGD_2 were shown to be synthesized in much smaller quantities.

Removal of the urothelium by rubbing reduced the amounts of prostanoids as seen in a thin layer chromatogram (Fig. 1), particularly 6-keto PGF_{1alpha}. Fig. 2 shows the presence of radioactivity in the products 6-keto PGF_{1alpha} and TXB_2 synthesized by intact and rubbed ureter from ^{14}C arachidonic acid. A 50% reduction in the synthesis of 6-keto PGF_{1alpha} could be shown to occur after removal of the urothelium. This change was statistically significant when compared with the intact situation, the reduction in TXB_2, however was

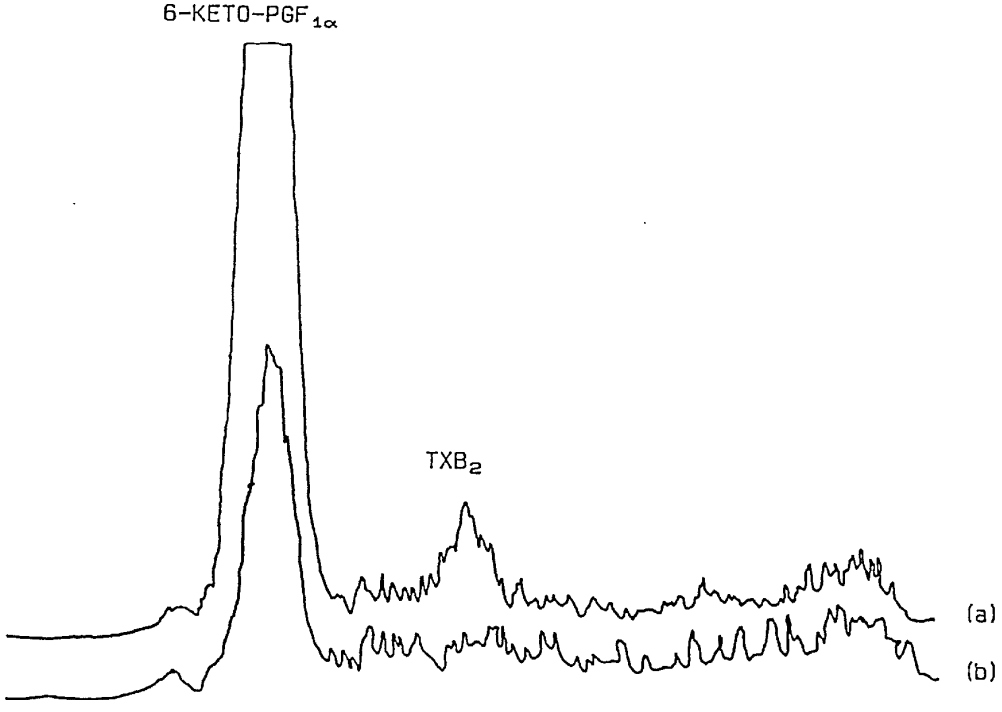

Fig. 1 Thin layer radiochromatogram scan of reaction products synthesized from ^{14}C arachidonic acid by both whole (**a**) and urothelium-removed (rubbed) (**b**) ureter

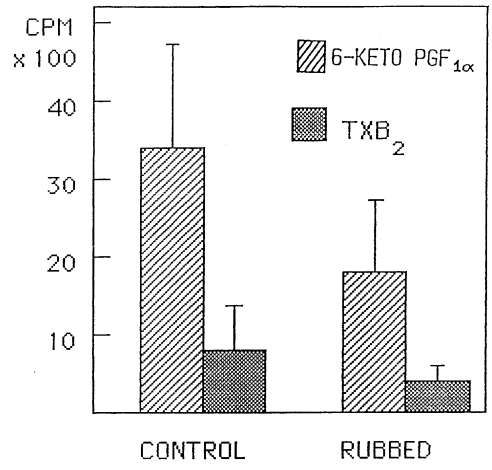

Fig. 2 Reaction products of 6-keto PGF_{1alpha} and TXB_2, synthesized by intact and urothelium-removed (rubbed) ureter from ^{14}C arachidonic acid. A 50% reduction in the synthesis of 6-keto PGF_{1alpha} was observed after rubbing the ureter. No significant difference was observed in the synthesis of TXB_2 between control and rubbed ureter (total counts per min × 100)

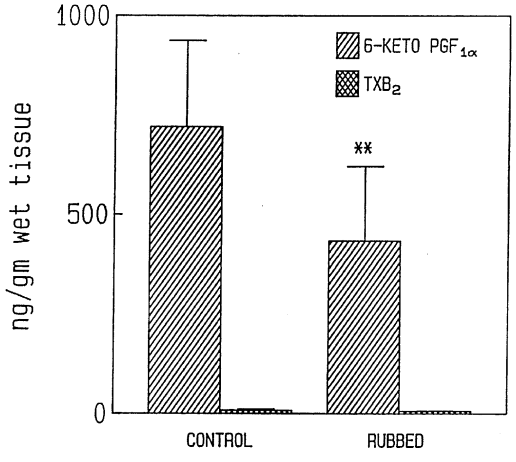

Fig. 3 Verification of radiometric assay results by radioimmunoassay carried out on the extracts from the incubation mixtures of both whole and rubbed ureter in the presence of arachidonic acid (100 mM). Again there was a significant difference in the synthesis of 6-keto PGF_{1alpha} by intact and rubbed ureter but no effect on the synthesis of TXB_2

not significant. Quantitative verification of radiometric assay was performed by radioimmunoassay measuring prostanoid synthesis of the extracts from the incubation mixtures. This analysis is displayed in Fig. 3 and again shows that rubbing significantly reduced only prostacyclin synthesis (as 6-keto PGF_{1alpha}) but not that of TXB_2.

The effect of indomethacin on synthesis by intact and rubbed ureter is demonstrated in Fig. 4. Here an inhibition of prostanoid synthesis by indomethacin was observed in both types of preparation at a concentration of 250 nM indomethacin.

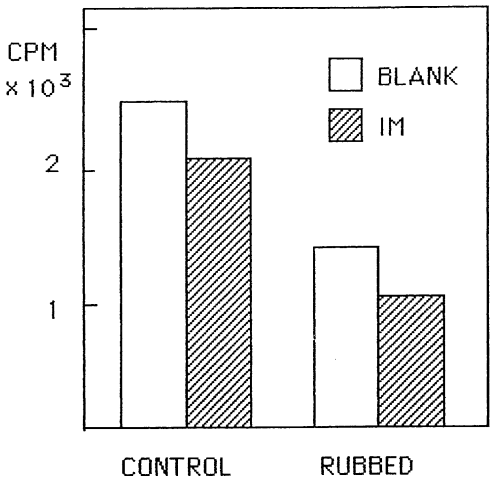

Fig. 4 The effect of indomethacin (IM, 250 nM) on prostanoid synthesis as expressed by counts per min (CPM) by intact and rubbed ureter (^{14}C, 6-keto PGF_{1alpha})

Discussion

The finding of 6-keto PGF_{1alpha}, the metabolite of prostacyclin being the major prostanoid produced by the ureter, is in line with our previous findings in the whole ureter [3, 12]. The reduced production after removal of the urothelium seems to be solely related to the synthetic capacity of the epithelium and not to a reduction in total tissue mass. This is because the epithelial layer of the ureter only constitutes a small part of the wall and the rubbing procedure preferentially affected 6-keto PGF_{1alpha} synthesis but not TXB_2. If prostanoid production were equally distributed in the different layers of the ureter one would have expected the same reduction in all types of prostanoids, but this was not the case.

Synthesis of prostanoids in each tissue depends on the complement of enzymes present and their relative abundance. Some tissues are able to synthesize the whole range of products whereas others, such as endothelial cells, contain primarily prostacyclin synthetase [8]. One could speculate that the traumatic procedure of rubbing the ureter in order to remove endothelial cells might have given rise to induction of a COX-2 enzyme but that did not seem to be the case, otherwise we could have expected an overall rise in prostanoid production. Moreover, COX-2 induction usually takes place only after exposure to inflammatory mediators, cytokines and oncogenes [7].

To our knowledge this is the first paper which explores the regional characteristics of prostanoid synthesis in the ureter. The finding of a preferential synthesis of prostacyclin in the urothelium is similar to the situation in blood vessels where prostacyclin, or rather its metabolite 6-keto PGF_{1alpha}, is predominantly produced by the inner lining of the vessel wall, the endothelium [7]. When speculating on the functional role

of this finding it seems likely that this is connected with an antithrombotic function of prostacyclin, thereby preventing obstructing clot formation in the ureter. This mechanism acts in concert with the intrarenal synthesis of the plasminogen activator urokinase which is excreted in the urine [6] and tissue plasminogen activator released by the ureter [9].

Another aspect of the functional role of prostanoids may concern their prokinetic action in the ureter, maintaining coordinated rhythmic peristaltic contractions which are of functional importance for the propulsion of urine towards the bladder. In previous studies on isolated ureters we found that prostanoids are of critical importance for proper peristaltic motility since inhibition of their synthesis by non-steroidal anti-inflammatory drugs and corticosteroids blocks smooth muscle pacemaker activity and conduction of impulses [4, 5, 10–12].

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