Tubular prostaglandin E₂ production and its role in urinary hypotonicity after release of ureteral occlusion in the rat

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SUMMARY

1. In order to explore the involvement of endogenous prostaglandin E₂ (PGE₂) in the urine concentration defect after ureteral occlusion, PGE₂ production by isolated collecting ducts in vitro and effects of indomethacin on urine osmolality in vivo were examined.

2. Twenty-four hours ureter obstruction caused increased PGE₂ production by the medullary collecting ducts, which was maintained at a high level on the day after release of obstruction (0.8±0.2 pg/mm normal, 8.1±0.9 pg/mm 24 h obstruction, and 6.6±1.0 pg/mm post-obstruction, mean ± SEM). An enhanced PGE₂ production was also observed for papillary collecting duct on the day after release of 24 h ureteral occlusion (3.9±0.5 pg/mm normal and 7.7±1.2 pg/mm post-obstruction).

3. Administration of indomethacin to the unilateral post-obstructive rats slightly raised the urine osmolality of the post-obstructed kidney (from 339±17 to 390±22 mosmol/kg H₂O), while it had a greater effect on the contralateral intact kidney (from 1569±138 to 2567±198 mosmol/kg H₂O).

4. Our data may indicate that the urine concentration defect after 24 h ureteral occlusion is ascribable mainly to a mechanism other than increased endogenous PGE₂.

Key words: cell injury, collecting duct, indomethacin, postobstructive concentration defect, thin descending limb of Henle.

Abbreviations: MCD, medullary collecting ducts; PCD, papillary collecting ducts; PGE₂, prostaglandin E₂.

INTRODUCTION

From a number of recent studies on prostaglandin synthesis and its role in regulation of renal functions, it has been established that the main endogenous tubular prostaglandin in normal kidney is E series [1–3], and that prostaglandin E₂ (PGE₂) inhibits vasopressin-mediated events in the medullary thick ascending limb of Henle's loop and collecting ducts in rat and rabbit [4, 5]. An increase in renal prostanoid synthesis has been observed during ureteral obstruction [6–8], and an immunohistochemical study by Smith & Bell shows an enhancement of cyclo-oxygenase activity in collecting ducts of the obstructed kidney [9]. On the other hand, a urine concentration defect remains after release of unilateral ureteral obstruction [10–12]. The most likely explanations for this defect are vasopressin resistance in collecting ducts and impairment of sodium chloride reabsorption in diluting segments, as clearly shown in Hanley & Davidson [13] with an isolated tubule perfusion technique.

These observations have led to a hypothesis that urine hypotonicity and vasopressin resistance after ureteral occlusion may be ascribed to PGE₂ (see [14]). However, it is unknown whether an increase in PGE₂ production is maintained as long as the urine concentration defect remains after the release of unilateral ureteral occlusion. Moreover, there is no direct evidence which demonstrates a correlation between PGE₂ synthesis and the urine concentration defect after release of ureteral occlusion.

To assess the contribution of PGE₂ to the urine concentration defect, we measured PGE₂ production by collecting ducts isolated from rat kidney during and after release of ureteral occlusion. Furthermore, the effect of indomethacin, an inhibitor of prostaglandin synthesis, on the urine osmolality was examined after release of the ureteral occlusion.

METHODS

Animal preparations

Wistar male rats, weighing 180–280 g, were used. Rats were fed normal chow and drank tap water ad libitum. Under ether anaesthesia, the lower quarter of the left ureter was completely obstructed by ligation with silk (5-0) for 4 h or 24 h. For relief of the obstruction, the left
ureter was catheterized with a tapered PE50 tubing just above the portion ligated, and the tubing was led to the back. The rat was allowed to recover from anaesthesia, and had free access to food and water during the next 24 h. Using these animals, the following two studies were carried out.

**PGE₂ production in medullary collecting ducts (MCD) and papillary collecting ducts (PCD)**

The following solutions were used in this study. ‘Collagenase solution’ consisted of 0.2% collagenase (type 1, Sigma), 0.1% bovine serum albumin (Sigma) and 20 units of heparin/ml in Krebs–Ringer bicarbonate solution with 8.3 mmol/l glucose, pH 7.4 at 30°C. ‘Incubation medium’ was Hank’s solution with 15 mmol/l Hepes and 8.3 mmol/l glucose. The concentration of calcium was 1 mmol/l. ‘Dissection medium’ consisted of incubation medium containing 0.025% fatty acid free bovine serum albumin (Sigma).

Rats were anaesthetized with sodium pentobarbital (50 mg/kg, intraperitoneally). The preparation of the renal slices and the dissection of MCD and PCD were performed as previously reported [5]. In short, after perfusion of the left kidney in situ with collagenase solution, 0.5–1.0 mm thick corticopapillary slices were prepared and incubated in the collagenase solution at 30°C for 30 min. MCD and PCD were dissected out by hand in dissection medium under a stereo-microscope. The length of isolated tubules was measured with a graticule eyepiece, and three to five tubules (1–4 mm) were transferred with 10 μl of dissection medium into a polypropylene tube (12 mm × 75 mm). Fresh incubation medium (90 μl) with or without the addition of 40 μmol/l indomethacin was given. The polypropylene tubes were capped. After incubation for 30 min at 37°C, 20 μl of 0.2 mol/l HCl was added to pH 3.0 and the solution was mixed well. PGE₂ was extracted twice with 0.7 ml of ethyl acetate, dried under N₂ gas, and kept at −80°C until assay. Arachidonic acid was not added exogenously to the incubation medium, because the purpose of the present study was to determine basal production using endogenous substrates, but not maximal activity using exogenous substrates.

PGE₂ was measured with a [¹²⁵I]PGE₂ radioimmunoassay kit (New England Nuclear, Boston, MA, U.S.A.). In our laboratory, this assay has an intra-assay coefficient of variation of 7.6% and an inter-assay coefficient of 10.8%. Recovery of [³H]PGE₂ by ethyl acetate extraction was 97–100%. The detection limit for PGE₂ was 0.25 pg per assay tube. The values were expressed as pg 30 min⁻¹ mm⁻¹ of tubular length. Because small amounts of immunoreactive PGE₂ were detected in the 0 min incubation (<0.5 pg/min), net production during the 30 min incubation was obtained by subtraction of the 0 min value in each experiment.

**Clearance study**

Clearance studies were performed on the day after release of 24 h unilateral ureteral occlusion, with the animals in a restraining cage under unanaesthetized conditions. Urine was collected through a PE50 catheter in the left ureter for the obstructed kidney, and in the bladder for the contralateral kidney. Blood pressure was monitored and blood samples were taken for measurement of packed cell volume, plasma inulin concentration and plasma sodium concentration, via a femoral arterial catheter. To avoid body fluid losses, 0.9% NaCl was given at a rate of 20 μl/min via a femoral vein catheter (PE10 tubing). When the urine flow rate was stabilized (usually 120 min after surgery for cannulation), urine was collected for a basal period (30 min). Indomethacin was administered as a priming dose of 5 mg kg⁻¹ body weight at a rate of 0.047 mg/min, followed by continuous infusion at 6 mg h⁻¹ kg⁻¹ body weight. This dose is known to depress the urinary excretion of PGE₂ by 95% [15]. Thirty minutes after initiating a continuous infusion of indomethacin, a 30 min experimental period was started. Plasma and urine inulin concentrations were measured by the Anthron method [16], and sodium concentrations were determined with an ion electrode (Orion Research, U.S.A.). Urine osmolality was determined with a vapour pressure osmometer (Shimazu Instr., Kyoto).

**Statistical analysis**

For statistical analysis, Student’s t-tests were used, and the values were expressed as means ± SEM.

**RESULTS**

**PGE₂ production by MCD and PCD in vitro**

As shown in Fig. 1, tubular PGE₂ production was linear with tubular length. In normal kidney PGE₂ production per tubular length (mm) was 0.8 ± 0.2 pg/30 min for MCD and 3.9 ± 0.5 pg/30 min for PCD. Indomethacin (40 μmol/l) decreased PGE₂ production from 3.6 to 0.6 pg 30 min⁻¹ mm⁻¹ in PCD (n = 3, P < 0.001), and from 0.8 to 0.1 pg 30 min⁻¹ mm⁻¹ in MCD (n = 5, P < 0.001).

The amount of PGE₂ produced by MCD was increased by 4 h ureteral obstruction (5.5 ± 1.8 pg/mm, n = 5, Table 1), and tended to become greater with elongation of the obstruction period (8.1 ± 0.9 pg/mm, n = 6). On the day after release of 24 h obstruction, PGE₂ production by MCD remained at a high level (6.6 ± 1.0 pg/mm, n = 8). PCD isolated from 4 h obstructed kidney produced PGE₂ in the same quantity as PCD from normal kidney (3.1 ± 0.9 and 3.9 ± 0.5 pg/mm, respectively). Twenty-four hour obstruction stimulated PGE₂ production by PCD (7.5 ± 3.4 pg/mm), but this value was not statistically significant. On the day after relief of 24 h obstruction, PCD isolated from the ipsilateral kidney produced 7.7 ± 1.2 PGE₂ pg/mm (P < 0.005 vs normal).

**Effects of indomethacin on renal functions**

Glomerular filtration rate was far lower in the obstructed kidney than in the contralateral kidney, and was not affected by indomethacin administration in either
kidney. These findings are in agreement with previous data [17].

Fractional water excretion during the basal period was higher in the obstructed than in the contralateral kidney (4.0 ± 1.1, and 0.6 ± 0.1%, respectively, Table 2). During indomethacin infusion, fractional water excretion was depressed in both obstructed and contralateral kidneys (2.1 ± 0.9 and 0.3 ± 0.1%, respectively). The absolute magnitude of this decrease in fractional water excretion was somewhat larger in the obstructed than in the contralateral kidney (1.9 ± 0.5 vs. 0.3 ± 0.1%, P < 0.05). Fractional sodium excretion was also decreased by indomethacin in both kidneys in the individual rats, but due to a wide range of variation this did not achieve statistical significance. The doses used in our experiments might not be the obstructed kidney and the contralateral kidney was 339 ± 17 and 1569 ± 138 mosmol/kg H₂O, respectively (P < 0.001, n = 5). As expected, urine osmolality in the contralateral kidney was increased markedly by indome-
thacin (2567 ± 198 mosmol/kg H₂O, P < 0.005). In the obstructed kidney, however, urine osmolality was only slightly affected by indomethacin administration (390 ± 22 mosmol/kg H₂O), and it did not rise even to the basal level in the untouched contralateral kidney in the same animal.

**DISCUSSION**

The collecting duct is an important site of tubular PGE₂ production in normal kidney [1, 18]. In recent experiments using isolated nephron segments, it was found that collecting ducts from the outer medulla can synthesize more PGE₂ than those from cortex [18, 19]. Prostaglandin is also synthesized in PCD cells [1, 2], although the contribution to renal PGE₂ production has not been determined. The present results demonstrate that the papillary portion of the collecting ducts also exhibits an extensive formation of PGE₂ that is four to five times greater than that of the outer medullary portion. From these observations, it is likely that there may be a PGE₂ concentration gradient from cortex to papilla in normal kidney.

PGE₂ synthesis has been shown to be stimulated in slices from ureter-obstructed kidneys [7, 8]. The sites responsible for this increment were immunohistochemically identified as endothelial cells of renal arterioles, interstitial cells and collecting duct cells [9]. However, there has not been any quantitative study to determine PGE₂ produced from endogenous substances. The present study measured the increased PGE₂ formation in collecting ducts of the obstructed kidney. Furthermore, on the day after release of 24 h obstruction, both MCD and PCD isolated from rat kidney exhibited increased generation of PGE₂, a finding supporting the possibility that in hydronephrotic kidney the enhancement of endogenous PGE₂ production would be prolonged after relief of the obstruction [14]. This notion is consistent with previous data which show accelerated activities of cyclooxygenase prepared from hydronephrotic rabbit kidney [6]. This increment was more prominent in MCD than in PCD both during and after relief of the obstruction: a response to ureteral occlusion on the PGE₂ production in PCD was slow and small. The difference between PCD and MCD may reflect sensitivity to an elevated intraluminal pressure after urinary tract obstruction. Alternatively, since PCD is thought to be usually exposed to a more hypoxic environment than MCD with respect to blood supply, PCD may

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**Fig. 1. PGE₂ production as a function of tubular length.**

Abscissa: length of papillary collecting ducts used (n = 11). Ordinate: amount of PGE₂ produced by each sample during a 30 min incubation period. Linear regression equation: y = 5.2x - 2.4, r = 0.787.

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**Table 1. PGE₂ production in MCD and PCD isolated from normal, obstructed or post-obstructed kidney**

<table>
<thead>
<tr>
<th>Segment</th>
<th>Normal</th>
<th>4 h obstructed</th>
<th>24 h obstructed</th>
<th>Post-obstructed</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCD</td>
<td>0.8 ± 0.2 (10)</td>
<td>5.5 ± 1.8 (5)*</td>
<td>8.1 ± 0.9 (8)*</td>
<td>6.6 ± 1.0 (8)*</td>
</tr>
<tr>
<td>PCD</td>
<td>3.9 ± 0.5 (11)#</td>
<td>3.1 ± 0.5 (5)</td>
<td>7.5 ± 3.4 (5)</td>
<td>7.7 ± 1.2 (7)*</td>
</tr>
</tbody>
</table>

Values are means ± SEM with the number of animals shown in parentheses. Statistical significance:

*P < 0.005 vs normal; †P < 0.01 vs MCD.
be more resistant to the tissue ischaemia [12] seen in ureteral occlusion.

As prostaglandins have an inhibitory effect on the vasopressin-mediated urine concentration mechanism in normal collecting ducts [4], it is likely that the enhanced PGE₂ production in the obstructed kidney may play an important role in the urine concentration defect after release of unilateral ureteral occlusion. Indeed, a role for PGE₂ in the pathophysiology of the ureter-obstructed kidney has been suggested (see [14]). To further investigate the relation between PGE₂ and urine hypotonicity, we examined the effect of indomethacin on urine osmolality after release of 24 h unilateral ureteral occlusion. Unexpectedly, the urine concentration defect was not rectified by indomethacin, which only slightly raised the urine osmolality. According to a report by Buergert et al. [11], filtering nephrons 90 min after release of unilateral ureteral occlusion represent 30-40% of the total nephrons. The doses used in our experiments might not be high enough to suppress prostaglandin synthesis in non-filtering tubules. Nevertheless, fractional water excretion, but not urine osmolality, of the obstructed kidney was markedly affected by indomethacin (Table 2). In agreement with the present study in vivo, Campbell et al. [20] recently reported, using perfusion of cortical collecting tubules of 4 h obstructed rabbit kidney in vitro, that vasopressin resistance to hydraulic water permeability and volume flux was not restored by 10 μmol/l indomethacin.

It may be postulated that both vasopressin resistance and exaggerated PGE₂ synthesis might be caused by a common factor(s). One of the candidates may be the calcium ion. PGE₂ production in intact cells is dependent on the intracellular calcium and/or calcium influx [21]. Also, the actions of vasopressin on the target cells appear to be modulated by intracellular calcium [22]. If cell membranes of collecting ducts in the obstructed kidney become highly permeable to calcium ions, it might be possible that the collecting ducts show both the enhanced PGE₂ synthesis and vasopressin resistance. Furthermore, in general, increased intracellular calcium ion could be a trigger that is followed by more profound cell injury, which would not be promptly reversed by any agents [23]. Thus, in the chronic stage of hydroureterohydronephrosis, vasopressin unresponsiveness may be indomethacin resistant. It is suggested that in our experimental model, the urine concentration defect observed after release of 24 h unilateral ureteral occlusion may be ascribed mainly to some mechanism(s) other than the increased production of PGE₂ per se.

It has been noted in normal rat that inhibitors of prostaglandin synthesis decrease medullary blood flow [24] and increase papillary solutes concentration [25, 26], and thereby would enhance the water reabsorption across the thin descending limb of Henle’s loop and collecting ducts. As described above, the obstructed kidney with vasopressin resistance in collecting ducts shows an increase in fractional water reabsorption without a change in glomerular filtration rate after indomethacin administration, a finding which suggests that indomethacin-induced water reabsorption in the obstructed kidney.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Control</th>
<th>Obstructed</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFR (ml/min)</td>
<td>1.24 ± 0.34</td>
<td>0.72 ± 0.48</td>
</tr>
<tr>
<td>Uₙ⁺ (mmol/kg H₂O)</td>
<td>1.31 ± 0.37</td>
<td>0.15 ± 0.28</td>
</tr>
</tbody>
</table>

Table 2. Clearance data before and during indomethacin treatment after release of 24 h unilateral ureteral occlusion.

Abbreviations: GFR, glomerular filtration rate; Uₙ⁺, sodium excretion; n = 5.

Significance was assessed by paired Student's t-test; before vs during; p < 0.05.

Post-obstructed kidney

<table>
<thead>
<tr>
<th>Control</th>
<th>Obstructed</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFR (ml/min)</td>
<td>1.34 ± 0.17</td>
</tr>
<tr>
<td>Uₙ⁺ (mmol/kg H₂O)</td>
<td>1.30 ± 0.30</td>
</tr>
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</table>

Contralateral kidney

Experiments were performed in five rats.
would take place in the thin descending limb. In addition, the finding that the absolute increment of fractional water reabsorption during indomethacin infusion is somewhat larger in the obstructed than in the contralateral kidney, implies that the amounts reabsorbed in the thin descending limb may be larger in the former than in the latter. If this is the case, accelerated water excretion in the obstructed kidney would be, at least in part, due to enhanced PGE₂ synthesis.

Regulation of thin descending limb function in vivo might be secondary to a decrease in medullary solutes concentration. A direct action of PGE₂ on this segment, however, may be possible. We previously reported the existence of PGE₂-sensitive adenylate cyclase activity in the thin descending limb [27, 28]. This enzyme was activated by low concentrations of PGE₂ (3.3–33 nmol/l), so that the functions of this segment might be influenced by PGE₂ released from collecting duct and interstitial cells, or from cells of the thin descending limb themselves of the obstructed kidney [9, 18]. Further studies are required to elucidate the mechanisms of the antidiuresis seen after indomethacin administration in the obstructed kidney.

Other effects (for example, on intrarenal circulation) of the increased PGE₂ in the obstructed kidney would be possible, but cannot be defined from the present study. In summary, the present experiment suggests that enhanced synthesis of PGE₂ after 24 h ureteral occlusion may not be a main cause of the urine hypotonicity seen.

ACKNOWLEDGMENT
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REFERENCES