Distribution of Prostaglandins E₂ and 6-Keto-F₁₅α Production in Dog Kidneys

Antonia Refoyo, Rodney J. Bolterman, Michael D. Bentley, Mary J. Fiksen-Olsen, Sharon M. Sandberg, and J. Carlos Romero

Little is known about the distribution of prostaglandin E₂ (PGE₂) and prostacyclin (PGI₂) production in the canine kidney. To determine the basal and stimulated profiles of PGE₂ and PGI₂ production along the corticomedullary axis of the dog kidney, a slice (0.5 mm thick, 10–50 mg) was obtained from six equally spaced zones along the axis (zone 1, medullary crest; zones 2 and 3, inner medulla; zone 4, outer medulla; and zones 5 and 6, cortex) and was divided into equal halves. One half of the slice was incubated with Krebs-Ringer buffer containing arachidonic acid (6.6×10⁻⁶ M), bradykinin (9.4×10⁻⁶ M), or indomethacin (10⁻⁵ M), whereas the remaining half of each slice was similarly incubated in Krebs-Ringer buffer alone. The production of PGE₂ and 6-keto-PGF₁₀α (the stable metabolite of PGI₂) was determined by radioimmunoassay. Under basal conditions, both PGE₂ and 6-keto-PGF₁₀α were highest in the innermost zones of the inner medulla (PGE₂, 3,328±549 pg/mg; 6-keto-PGF₁₀α, 1,611±129 pg/mg) and decreased exponentially to low levels in the cortex (PGE₂, undetectable; 6-keto-PGF₁₀α, 13±2 pg/mg); this production was inhibited by indomethacin. Arachidonic acid significantly increased the production of PGE₂ in all zones of the kidney and the production of 6-keto-PGF₁₀α only in zones 3–6. Bradykinin significantly stimulated production of PGE₂ in zones 1, 3, and 4 and 6-keto-PGF₁₀α in zones 4–6, suggesting that in the inner medulla, unlike the cortex, 6-keto-PGF₁₀α production was saturated under basal conditions and could not be stimulated further. PGE₂ production, on the other hand, was increased throughout all zones of the kidney with excess substrate (arachidonic acid) but was most sensitive to stimulation by bradykinin in the inner medulla. (Hypertension 1990;15(suppl I):I-107–I-111)

The kidney has a high capacity for the synthesis of prostaglandins by a cascade of enzymatic steps.1,2 Several of these prostaglandins, such as prostaglandin E₂ (PGE₂) and prostacyclin (PGI₂), are thought to have modulatory effects on renal circulation, natriuresis, and diuresis. For instance, maneuvers that increase the production of PGE₂ and PGI₂ in the dog kidney also induce natriuresis, diuresis, and vasodilatation.1,2 In the kidney, these prostaglandins can be synthesized within discrete anatomic compartments so that specific effects on renal function are produced.3 In vivo studies in dogs have shown that intrarenal infusion of either bradykinin (BK) or arachidonic acid (AA) increased both the urinary excretion and venous outflow of PGE₂ but only increased the venous outflow of 6-keto-PGF₁₀α, the stable metabolite of PGI₂.4 It has been shown in the rat and rabbit that there is a gradient in the synthesis of PGE₂ along the corticomedullary axis, with maximal production in the renal papilla.5,6 Less is known about the anatomic profile of PGI₂ synthesis. Although isolated structures such as glomeruli, inner medullary collecting ducts, and endothelial cells of larger renal blood vessels produce PGI₂,5,6 these structures are each located in different anatomic regions of the kidney. Whereas the synthesis of PGI₂ appears to be greater in the medulla (inner and outer) than in the cortex,5,6 one study using rat kidney tissue6 has shown the maximal level of PGI₂ synthesis is in the outer medulla rather than in the papilla. In the dog kidney, Satoh and Satoh7 reported that the major prostaglandin produced by renal medullary and cortical microsomal preparations was not PGE₂ but rather the stable metabolite of PGI₂, 6-keto-PGF₁₀α. These data imply possible species differences of the dog from the rat and rabbit. In contrast, by using slices of renal tissue, Okahara et al8 found that both PGE₂ and PGI₂ were abundantly produced by the inner medulla of the dog kidney in vivo.9 Hence, the cortex has a higher capacity for the synthesis of PGI₂ than the medulla.
dog kidney. This anatomic distribution of PGE\(_2\) and PGI\(_2\) production, however, was studied only under basal conditions in the canine kidney. In the present study, we have measured the production of PGE\(_2\) and 6-keto-PGF\(_{1\alpha}\) along the corticomedullary axis of the canine kidney during basal conditions, stimulation with BK and AA and inhibition with indomethacin.

**Methods**

Kidneys were removed from 22 healthy mongrel dogs (either sex, 18–30 kg) that had been anesthetized with sodium pentobarbital (30 mg/kg i.v.). After flushing the blood from each kidney with cold saline 0.9% (4°C), a core of tissue was obtained by removing the cranial and caudal poles and both sides of the kidney at the pelvic fornices. This core, containing the medullary crest, inner and outer medulla, and cortex, was placed in ice-cold (4°C) modified Krebs-Ringer buffer (KRB) (mM): (NaCl 118, dextrose 11, KCl 4.7, MgSO\(_4\) 1.2, KH\(_2\)PO\(_4\) 1.2, NaCO\(_3\) 25, CaCl\(_2\) 2.5 and pH 7.4).

After cooling, six slices (0.5-mm thick) at equally spaced intervals along the axis of the core (corticomedullary axis) were obtained with a Thomas-Stadie-Riggs tissue slicer (Thomas Scientific, Swedesboro, New Jersey). The spacing of the intervals was such that slice 1 was at the medullary crest, slice 2 at the midinner medulla, slice 3 at the outer part of the inner medulla, slice 4 at the outer medulla, slice 5 at the deep cortex, and slice 6 at the outer cortex. Slices were preincubated for 30 minutes at 37°C in 1 ml KRB (continuously gassed with 95% O\(_2\) and 5% CO\(_2\)) and washed with 2 ml KRB (37°C). All liquid was thoroughly aspirated from each tube before the final 30 minutes' incubation in 1 ml of KRB, KRB with indomethacin, KRB with AA or KRB with BK. Samples were obtained from each tube before starting the incubation period (0 minutes) and at the end of the incubation period (30 minutes) for subsequent radioimmunoassay of PGE\(_2\) and 6-keto-PGF\(_{1\alpha}\). The tissue slices were blotted and weighed (cortical slices, 50–70 mg; medullary slices, 18–30 mg). The slices were then homogenized in 10% trichloroacetic acid and their protein content determined with BCA (a chemical assay commercially available from Pierce Chemical Company, Rockford, Illinois). Protein content for the cortical slices was 137.12±6.30 \(\mu\)g protein/mg wet tissue wt and 37.77±2.40 \(\mu\)g protein/mg wet tissue wt for the medullary slices. The production of prostaglandin in the 30-minute incubation period was calculated from the difference of prostaglandin concentrations at 0 and 30 minutes divided by either the tissue weight or the protein content of the slice.

In the three experimental groups, each of the six slices in the core was divided into equal halves before preincubation. One half of the slice was used to determine the stimulated (BK, AA) or inhibited (indomethacin) production of prostaglandin, and the remaining half was incubated in KRB alone as a basal control. In the first group \((n=8)\), 9.4x10\(^{-6}\) M BK (triacetate salt, Sigma Chemical Co., St. Louis, Missouri) was included in the incubation medium in the first group, whereas in the second group \((n=7)\), 6.6x10\(^{-4}\) M AA (NuCheck Prep, Elysian, Minnesota) was added. In the third group \((n=7)\), 10\(^{-2}\) M indomethacin (Merck L Co., West Point, Pennsylvania) was included in both the preincubation and the incubation media. The determination of PGE\(_2\) and 6-keto-PGF\(_{1\alpha}\) was performed by radioimmunoassay (antibody from Advanced Magnetics Inc., Cambridge, Massachusetts). PGE\(_2\) antibody cross-reactivity is 100% with PGE\(_1\).

All values have been expressed as mean±SEM. Analysis of variance (randomized block design) was used to detect differences in prostaglandin production among the six zones. The Student's paired \(t\) test was used to detect differences in prostaglandin production between the treated half of the slice and the untreated basal control half. Differences were considered significant if \(p\) values were less than 0.05.

**Results**

Microscopic examination of the tissue confirmed that zone 1 was the medullary crest having large collecting ducts and numerous interstitial cells; zone 2 was the mid-inner medulla having smaller collecting ducts and numerous interstitial cells; zone 3 was the outer part of the inner medulla with even smaller collecting ducts; zone 4 was the outer medulla with thick ascending limbs and vascular bundles; zone 5 was the deep cortex with abundant renal corpuscles, convoluted tubules and medullary rays; and zone 6 was the outer cortex with smaller and infrequent medullary rays.

Analysis of variance indicated that there were significant differences in the production of both PGE\(_2\) and 6-keto-PGF\(_{1\alpha}\) among the six zones of the kidney under basal and stimulated (BK and AA) conditions (Tables 1 and 2). In general, there was a high production of both prostaglandins at the crest of the inner medulla and an exponential decrease toward the cortex. This gradient was observed regardless of whether prostaglandin production was expressed per unit wet weight or per protein content of the slices.

Incubation of slices with 9.4x10\(^{-6}\) M BK significantly elevated the production of PGE\(_2\) above basal values in the medulla (zones 1, 3, and 4) but not in the cortex (Table 1). In contrast, the production of 6-keto-PGF\(_{1\alpha}\) was significantly stimulated in the outer medulla and cortex (zones 4–6) but not in the inner medulla (Table 2).

Incubation of slices with 6.6x10\(^{-4}\) M AA significantly elevated the production of PGE\(_2\) above basal values in all zones of the kidney (Table 1). The production of 6-keto-PGF\(_{1\alpha}\), however, was increased significantly only in zones 3–6 (Table 2).

In the presence of indomethacin, the production of both PGE\(_2\) and 6-keto-PGF\(_{1\alpha}\) was decreased significantly below basal levels (Tables 1 and 2).
Table 1. Production of Prostaglandin E₂ in Regions Along Corticomedullary Axis

<table>
<thead>
<tr>
<th>Zone</th>
<th>Treatment</th>
<th>Basal (pg/mg wet wt/30 min)</th>
<th>Treated (pg/mg wet wt/30 min)</th>
<th>Difference (pg/mg wet wt/30 min)</th>
<th>Basal (pg/mg protein/30 min)</th>
<th>Treated (pg/mg protein/30 min)</th>
<th>Difference (pg/mg protein/30 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zone 1 (crest of inner medulla)</td>
<td>AA</td>
<td>4,698 ± 1,445</td>
<td>6,506 ± 2,195*</td>
<td>3,807 ± 875</td>
<td>147.1 ± 54.6</td>
<td>304.3 ± 110.8*</td>
<td>157.2 ± 63.8</td>
</tr>
<tr>
<td></td>
<td>BK</td>
<td>3,016 ± 533</td>
<td>3,943 ± 676*</td>
<td>927 ± 311</td>
<td>89.9 ± 16.1</td>
<td>112.2 ± 29.0</td>
<td>22.3 ± 16.0</td>
</tr>
<tr>
<td></td>
<td>INDO</td>
<td>2,315 ± 591</td>
<td>358 ± 75*</td>
<td>-1,957 ± 572</td>
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</tr>
<tr>
<td>Zone 2 (mid-inner medulla)</td>
<td>AA</td>
<td>1,077 ± 210</td>
<td>1,784 ± 383*</td>
<td>707 ± 203</td>
<td>24.2 ± 4.4</td>
<td>42.9 ± 16.4</td>
<td>18.6 ± 16.1</td>
</tr>
<tr>
<td></td>
<td>BK</td>
<td>1,283 ± 255</td>
<td>1,564 ± 385</td>
<td>281 ± 195</td>
<td>29.5 ± 6.9</td>
<td>39.1 ± 7.1*</td>
<td>9.6 ± 3.4</td>
</tr>
<tr>
<td></td>
<td>INDO</td>
<td>524 ± 132</td>
<td>93 ± 19*</td>
<td>-431 ± 133</td>
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<tr>
<td>Zone 3 (outer part of inner medulla)</td>
<td>AA</td>
<td>161 ± 44</td>
<td>493 ± 127*</td>
<td>332 ± 91</td>
<td>3.5 ± 0.7</td>
<td>12.4 ± 4.2*</td>
<td>8.8 ± 3.8</td>
</tr>
<tr>
<td></td>
<td>BK</td>
<td>152 ± 30</td>
<td>579 ± 205*</td>
<td>428 ± 188</td>
<td>4.0 ± 0.8</td>
<td>15.6 ± 6.9</td>
<td>11.5 ± 6.6</td>
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<td></td>
<td>INDO</td>
<td>62 ± 20</td>
<td>13 ± 2*</td>
<td>-49 ± 21</td>
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<tr>
<td>Zone 4 (outer medulla)</td>
<td>AA</td>
<td>10 ± 6</td>
<td>129 ± 35*</td>
<td>119 ± 33</td>
<td>0.3 ± 0.2</td>
<td>1.3 ± 0.3*</td>
<td>0.9 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>BK</td>
<td>5 ± 2</td>
<td>21 ± 3*</td>
<td>16 ± 4</td>
<td>0.1 ± 0.1</td>
<td>0.3 ± 0.1*</td>
<td>0.3 ± 0.1</td>
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<tr>
<td></td>
<td>INDO</td>
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<tr>
<td>Zone 5 (deep cortex)</td>
<td>AA</td>
<td>. . .</td>
<td>120 ± 39*</td>
<td>120 ± 39</td>
<td>1.0 ± 0.4*</td>
<td>10.0 ± 0.4</td>
<td>1.0 ± 0.4</td>
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<tr>
<td></td>
<td>BK</td>
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<tr>
<td>Zone 6 (superficial cortex)</td>
<td>AA</td>
<td>. . .</td>
<td>51 ± 23</td>
<td>42 ± 24</td>
<td>0.9 ± 0.6</td>
<td>0.9 ± 0.6</td>
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<tr>
<td></td>
<td>BK</td>
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AA, arachidonic acid (6.6 x 10⁻⁴ M, n=6); BK, bradykinin (9.4 x 10⁻⁶ M, n=8); INDO, indomethacin (10⁻³ M, n=7).

*p<0.05 as compared with basal, paired t test.

Discussion

In the present study, we have shown that there is a gradient of both PGE₂ and 6-keto-PGF₁α production in the canine kidney, having high production of both prostaglandins at the crest of the inner medulla and an exponential decrease toward the cortex. The basal production of both PGE₂ and 6-keto-PGF₁α was blocked by indomethacin, indicating de novo synthesis and not spontaneous tissue washout or release. Similar results for the canine kidney have previously been described by Okahara et al¹⁸ who demonstrated that the basal production of PGE₂ and 6-keto-PGF₁α was considerably higher in the inner medulla than in the outer medulla and cortex. Unlike their study, however, the inner medulla in our study was further subdivided into three separate zones. The inner medullary values reported by Okahara et al¹⁸ corresponded to values between zones 2 and 3 in our study.

The heterogeneity of prostaglandin production reflects the heterogeneity of the renal tissue along the corticomedullary axis and the relative abundance of cell types capable of prostaglandin synthesis. Histochemistry and immunofluorescence studies have shown a high level of prostaglandin synthesis in vascular endothelium, glomerular mesangial cells, medullary collecting ducts, and interstitial cells.¹² The synthesis of specific prostaglandins has been demonstrated by cultures of these cells⁹,¹⁰ although such cultures can have different synthetic capabilities than those of parent cells in situ. Preparations of microdissected nephrons, blood vessels, and glomeruli have provided further information concerning the synthesis of various prostaglandins.²⁵⁶ The relative contribution, however, of less abundant cell types (e.g., medullary interstitial cells) that might have high synthetic capabilities for prostaglandin synthesis is not known.

When provided with a nonreceptor-mediated stimulus such as AA, the production of PGE₂ was increased in all zones of the kidney, implying that synthetic enzymes for PGE₂ are present throughout. In contrast, when slices were exposed to a receptor-mediated stimulus such as BK, a detectable increase in PGE₂ synthesis was seen in the medullary slices but not in cortical slices. These results suggest that BK receptors for PGE₂ synthesis might be located primarily in the inner medulla. Although similar results have been demonstrated in collagenase-dispersed cells from the cortex and medulla of rat kidneys, other studies with tissue from rabbit kidneys have shown BK stimulation of PGE₂ synthesis in cortical structures (e.g., cortical collecting ducts) as well as in medullary structures.¹¹¹² It is possible that PGE₂ was not stimulated to detectable levels despite the maximal concentration of BK and that the cortical slices were larger than medullary slices (see Methods) or, alternatively, the rate of PGE₂ degradation might...
PGI2 might be involved in cortical functions such as autoregulation of renal blood flow and filtration and the release of renin. Conversely, because the regulation of PGE2 production seems to be directed toward the medulla but can be further increased by AA throughout the kidney, PGE2 might be more involved in medullary and tubular functions such as the modulation of natriuresis and diuresis.

We have demonstrated high levels of both PGE2 and 6-keto-PGF1α in the inner medulla. AA increased the production of PGE2 in all zones of the kidney but the production of 6-keto-PGF1α was increased only in zones 3–6 and not in the inner part of the inner medulla (zones 1 and 2). In contrast, BK stimulated the medullary production of PGE2 (zones 1, 3, and 4) and cortical production of 6-keto-PGF1α (zones 4–6).

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**KEY WORDS** • prostaglandins • prostacyclin • kidney • indomethacin • arachidonic acid • bradykinin
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