Distribution of Prostaglandins E\textsubscript{2} and 6-Keto-F\textsubscript{1\alpha} 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 E\textsubscript{2} (PGE\textsubscript{2}) and prostacyclin (PGI\textsubscript{2}) production in the canine kidney. To determine the basal and stimulated profiles of PGE\textsubscript{2} and PGI\textsubscript{2} 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\textsuperscript{-7} M), bradykinin (9.4×10\textsuperscript{-4} M), or indomethacin (10\textsuperscript{-5} M), whereas the remaining half of each slice was similarly incubated in Krebs-Ringer buffer alone. The production of PGE\textsubscript{2} and 6-keto-PGF\textsubscript{1\alpha} (the stable metabolite of PGI\textsubscript{2}) was determined by radioimmunoassay. Under basal conditions, both PGE\textsubscript{2} and 6-keto-PGF\textsubscript{1\alpha} were highest in the innermost zones of the inner medulla (PGE\textsubscript{2}, 3,328±549 pg/mg; 6-keto-PGF\textsubscript{1\alpha}, 1,611±129 pg/mg) and decreased exponentially to low levels in the cortex (PGE\textsubscript{2}, undetectable; 6-keto-PGF\textsubscript{1\alpha}, 13±2 pg/mg); this production was inhibited by indomethacin. Arachidonic acid significantly increased the production of PGE\textsubscript{2} in all zones of the kidney and the production of 6-keto-PGF\textsubscript{1\alpha} only in zones 3–6. Bradykinin significantly stimulated production of PGE\textsubscript{2} in zones 1, 3, and 4 and 6-keto-PGF\textsubscript{1\alpha} in zones 4–6, suggesting that in the inner medulla, unlike the cortex, 6-keto-PGF\textsubscript{1\alpha} production was saturated under basal conditions and could not be stimulated further. PGE\textsubscript{2} 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)
dog kidney. This anatomic distribution of PGE₂ and PGI₂ production, however, was studied only under basal conditions in the canine kidney. In the present study, we have measured the production of PGE₂ and 6-keto-PGF₁α, 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, MgSO₄ 1.2, KH₂PO₄ 1.2, NaCO₃ 25, CaCl₂ 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 precultured for 30 minutes at 37°C in 1 ml KRB (continuously gassed with 95% O₂ and 5% CO₂) 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 (37°C). 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 PGE₂ and 6-keto-PGF₁α. 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 μg protein/mg wet tissue wt and 37.77±2.40 μg 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), 9.4×10⁻⁶ 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), 6.6×10⁻⁴ M AA (NuCheck Prep, Elyssian, Minnesota) was added. In the third group (n=7), 10⁻³ M indomethacin (Merck L Co., West Point, Pennsylvania) was included in both the preincubation and the incubation media. The determination of PGE₂ and 6-keto-PGF₁α was performed by radioimmunoassay (antibody from Advanced Magnetics Inc., Cambridge, Massachusetts). PGE₂ antibody cross-reactivity is 100% with PGE₁.

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 PGE₂ and 6-keto-PGF₁α 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 9.4×10⁻⁶ M BK significantly elevated the production of PGE₂ 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-PGF₁α was significantly stimulated in the outer medulla and cortex (zones 4–6) but not in the inner medulla (Table 2).

Incubation of slices with 6.6×10⁻⁴ M AA significantly elevated the production of PGE₂ above basal values in all zones of the kidney (Table 1). The production of 6-keto-PGF₁α, however, was increased significantly only in zones 3–6 (Table 2).

In the presence of indomethacin, the production of both PGE₂ and 6-keto-PGF₁α 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 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>8,506 ± 2,195*</td>
<td>3,807 ± 875*</td>
</tr>
<tr>
<td></td>
<td>BK</td>
<td>3,016 ± 533</td>
<td>3,943 ± 676*</td>
<td>927 ± 311</td>
</tr>
<tr>
<td></td>
<td>INDO</td>
<td>2,315 ± 591</td>
<td>358 ± 75*</td>
<td>-1,957 ± 572</td>
</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>
</tr>
<tr>
<td></td>
<td>BK</td>
<td>1,283 ± 255</td>
<td>1,564 ± 385</td>
<td>281 ± 195</td>
</tr>
<tr>
<td></td>
<td>INDO</td>
<td>524 ± 132</td>
<td>93 ± 19*</td>
<td>-431 ± 133</td>
</tr>
<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>
</tr>
<tr>
<td></td>
<td>BK</td>
<td>152 ± 30</td>
<td>579 ± 205*</td>
<td>428 ± 188</td>
</tr>
<tr>
<td></td>
<td>INDO</td>
<td>62 ± 20</td>
<td>13 ± 2*</td>
<td>-49 ± 21</td>
</tr>
<tr>
<td>Zone 4 (outer medulla)</td>
<td>AA</td>
<td>10 ± 6</td>
<td>129 ± 35*</td>
<td>119 ± 33</td>
</tr>
<tr>
<td></td>
<td>BK</td>
<td>5 ± 2</td>
<td>21 ± 3*</td>
<td>16 ± 4</td>
</tr>
<tr>
<td></td>
<td>INDO</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zone 5 (deep cortex)</td>
<td>AA</td>
<td>120 ± 39*</td>
<td>120 ± 39</td>
<td>0 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>BK</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>INDO</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zone 6 (superficial cortex)</td>
<td>AA</td>
<td>51 ± 23</td>
<td>42 ± 24</td>
<td>0.9 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>BK</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>INDO</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AA, arachidonic acid (6.6 × 10⁻⁴ M, n = 6); BK, bradykinin (9.4 × 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 al. 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 al. 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. The synthesis of specific prostaglandins has been demonstrated by cultures of these cells 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. 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. 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).

Acknowledgments

We are grateful to Paulette Peterson for the careful preparation of the manuscript and to Dr. Miguel Salom for his assistance with data analysis.

References

5. Farman N, Pradelles P, Bouvalet JP: PGE\textsubscript{1}, PGE\textsubscript{2}, PGF\textsubscript{2\alpha}, 6-keto-PGF\textsubscript{1\alpha} and TxB\textsubscript{2} synthesis along the rabbit nephron. *Am J Physiol* 1987;252:F53–F59

**KEY WORDS** • prostaglandins • prostacyclin • kidney • indomethacin • arachidonic acid • bradykinin
A Refoyo, R J Bolterman, M D Bentley, M J Fiksen-Olsen, S M Sandberg and J C Romero

Distribution of prostaglandins E2 and 6-keto-F1 alpha production in dog kidneys.

Hypertension. 1990;15:I107
doi: 10.1161/01.HYP.15.2_Suppl.I107

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1990 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/15/2_Suppl/I107

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/