Specific Regulation of Peptide-induced Renal Prostaglandin Synthesis*
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Repeated hourly stimulation of perfused rabbit hydronephrotic kidney (HNK) with a fixed dose of bradykinin resulted in a progressive (20-fold in 5 h) increase in prostaglandin (PG) synthesis. Inhibition of protein synthesis with cycloheximide during perfusion abolished the enhanced time-dependent responsiveness of the HNK to peptide stimulation, whereas pretreatment of rabbits with aspirin, which covalently inactivates cyclooxygenase, only temporarily suppressed the peptide response. Utilization of exogenous arachidonate bypasses the renal peptide receptor and acyl hydrolyase and the PG production by the fatty acid therefore reflects total (peptide-linked and peptide-independent) cyclooxygenase activity of the tissue. Arachidonate metabolism by renal microsomes or intact perfused HNKs was: (a) independent of perfusion time, (b) unchanged by perfusion with cycloheximide, and (c) abolished by aspirin pretreatment of the animal. In addition, cortical (but not medullary) microsomes obtained from HNKs possess more cyclooxygenase activity than microsomes prepared from contralateral (unobstructed) or normal kidneys. The striking quantitative differences between endogenous (i.e. peptide-activated) and exogenous arachidonate-induced PG synthesis are further accentuated by major qualitative difference in end product. The primary metabolite of endogenous arachidonate is PGE\textsubscript{2}, while exogenous arachidonate is converted to prostacyclini. Perfusion of the HNK apparently results in a selective, time-dependent induction of new protein synthesis (inhibited by cycloheximide) of a highly specific peptide (bradykinin or angiotensin)-linked cyclooxygenase. There is another much larger pool of renal cyclooxygenase, in hydrophobic or normal kidneys, which is irreversibly inhibited by aspirin and therefore does not undergo de novo synthesis during perfusion and is unaffected by cycloheximide. We previously demonstrated that peptide stimulation of an isolated perfused rabbit kidney removed 3 days after ureter obstruction, (hydronephrotic kidney, HNK) resulted in a marked enhancement of prostaglandin (PGE\textsubscript{2}) and thromboxane biosynthesis (1-3). Hourly administration of a fixed dose of bradykinin to an isolated perfused kidney removed from a hydronephrotic rabbit caused a time-dependent increase in the release of bioassayable PG\textsubscript{2} (4). The perfused, unobstructed contralateral kidney (CLK) releases only small amounts of PG in response to peptides in a time-dependent fashion. The time-dependent progressive increase in peptide-stimulated PG release was reversibly blocked by inhibition of RNA or protein synthesis (4). Aspirin, which covalently acetylates cyclooxygenase (5), was administered in vivo to hydronephrotic rabbits, and inhibited the initial bradykinin-induced PG release in the perfused kidney, but within 60 to 90 min of perfusion, there was a progressive bioassayable PGE\textsubscript{2} release in response to bradykinin which paralleled the non-aspirin-treated hydronephrotic control (4). In the aspirin-treated CLK and normal kidneys, peptide-stimulated PG release was inhibited and did not recover during perfusion.

Although the renal medulla has been advocated to be the major site of renal PG biosynthesis some cortical PG production has been detected (6, 7). Inhibition of cyclooxygenase activity in the perfused HNK increased basal perfusion pressure (renal resistance determinants are cortical (8)) and enhanced the renal vasoconstriction produced by angiotensin II (1). Furthermore, incubation of hydronephrotic microsomal preparations showed that both the cortex and medulla could synthesize PG and thromboxane (3).

In the present investigation we were interested in localizing the renal site of the de novo cyclooxygenase synthesis responsible for the time-dependent increase in PG production in the perfused hydronephrotic rabbit kidney. In addition we planned to compare total cortical and medullary microsomal cyclooxygenase activity, (i.e. metabolism of exogenous [\textsuperscript{14}C]-arachidonate) from perfused hydronephrotic kidneys obtained during different states of activation or inhibition of enzyme synthesis. However, much to our surprise, during these experiments we unmasked a profound difference in PG biosynthesis induced by exogenous or endogenous (i.e. peptide-stimulated release) and arachidonate metabolism which may, in fact, suggest an exquisite compartmentation of a cyclooxygenase specifically linked to activation of hormone receptors.

**MATERIALS AND METHODS**

Unilateral ureteral obstruction was carried out in New Zealand male white rabbits by a previously described procedure (1). After 3 days the animals were anesthetized (pentobarbital, 30 mg/kg) and heparinized (250 units/kg), and the renal arteries were cannulated. The kidneys were excised, placed in a warming jacket, and perfused at 10 ml/min with oxygenated (O\textsubscript{2}:CO\textsubscript{2}, 95%:5%) Krebs Henseleit buffer at 37°C. Renal venous effluent containing PG was acidified to pH 3.0 to 3.5 with 2 N formic acid and extracted twice with equal volumes of ethyl acetate and dried over anhydrous Na\textsubscript{2}SO\textsubscript{4}. The extract was vacuum-evaporated to dryness and reconstituted in a small volume of chloroform:methanol (2:1). The chloroform:methanol extract was evaporated to dryness under N\textsubscript{2} and reconstituted in saline (0.9% NaCl) prior to bioassay. The PGE\textsubscript{2}-like activity of the extracted samples was determined on smooth muscle assay organs (rat stomach strip and chick rectum) in comparison to standard PGE\textsubscript{2} doses. In the labeling experiments, the kidney was infused with 2 X 10\textsuperscript{7} cpm of [\textsuperscript{14}C]arachidonate (prepared as the sodium salt) over 20 min by methods previously described (9). The kidneys extracted to 90% of the label under these circumstances, which was largely incorporated into the renal phospholipids. The effluent from these kidneys was then extracted (see above) and subjected to thin layer chromatography using a solvent system of benzene:dioxane:acetic acid (97:2:1).
Cycloheximide was infused at the rate of 50 μg/min through the kidney. In the aspirin experiments, the rabbits were given 1 g/kg (orally) of aspirin 2 h prior to removal of the kidney for perfusion. 

\[ ^{[1-\text{H}]}	ext{Arachidonic acid (Amersham/Seear): specific activity, 55 mCi/mmol) was used. Cycloheximide, and acetylsalicylic acid were obtained from Sigma. Prostaglandin standards were a generous gift of Dr. John Pike of the Upjohn Co. }\]

**Microsomal Experiments**—The isolated rabbit kidneys were frozen upon completion of the perfusion experiment and were stored at \(-70\°C\) overnight. The frozen tissue was partially thawed and the cortex was separated from the medulla. The tissues were diced and homogenized in 3 volumes of 300 mM Tris buffer (pH 7.5) with a polytron and centrifuged at 8000 \(\times g\) for 15 min. The supernatant fraction was centrifuged at 100,000 \(\times g\) for 60 min. The microsomal pellet was resuspended in 100 mM phosphate buffer (pH 7.5) and the protein concentration was determined with fluorescamine. The medullary microsomes (300 μg of protein) were incubated (final volume, 1 ml) with \([^{3}C]\)arachidonic acid (300,000 cpm, 1 μg) in the presence of L-epinephrine (1.2 mM) and reduced glutathione (1 mM) at 37°C for 15 min. Cortical microsomes (600 μg of protein) were similarly incubated for 30 min. The reaction was stopped by adding 2 N formic acid and adjusting the pH to 3.0 to 3.5 and reaction mixture extracted twice with 2 volumes of ethyl acetate. The extracted lipids (with added unlabeled PG standards) were concentrated and chromatographed on silica gel G-thin layer plates (Brinkman) in a solvent system of benzene:dioxane:acetic acid (60:30:3). The plates were monitored on a Vangard radioisotope scanner, and the unlabeled PG standards were visualized with iodine vapor. The radioactive peaks were scraped and counted in scintillation mixture (Omnifluor-New England Nuclear in toluene) with a Beckman liquid scintillation counter. When renal microsomes (medulla or cortex) were prepared and tested immediately after perfusion, the primary detectable arachidonate metabolites obtained were PGF₂α and thromboxane B₂. However, when frozen tissue was employed the arachidonate products included PGE₂, PGF₂α, and PGD₂ but no thromboxane B₂.

**RESULTS**

**Peptide-stimulated PG Synthesis in Intact Perfused Hydronephrotic Kidneys**—Intact kidneys were removed from ureter obstructed (hydronephrotic kidney) animals and perfused with Krebs-Henseleit media for 5 h. The perfused kidneys were stimulated each hour with a fixed dose of bradykinin and 50 ml of the renal venous effluent was acidified and extracted with ethyl acetate. The concentrated extract was assayed for PGD₂-like biological activity on calibrated smooth muscle assay tissues (rat stomach strip and chick rectum). Hourly stimulation of the perfused HNK with a fixed dose (1 μg) of bradykinin resulted in a 20-fold increase in prostaglandin release during a 4-h perfusion period (Fig. 1). No such time-dependent change in PG production in response to peptide stimulation occurred in perfused contralateral unobstructed or normal rabbit kidneys (1). Continuous infusion of cycloheximide into the HNK resulted in a 90% inhibition (at the perfusion experiments (4). Continuous infusion of cycloheximide into the HNK resulted in a 90% inhibition (at the perfusion experiments (4). Continued infusion of cycloheximide into the HNK resulted in a 90% inhibition (at the perfusion experiments (4).

**Total Cyclooxygenase Activity in Microsomes**—The above intact kidney experiments suggest that the progressive enhancement of renal PG synthesis in the HNK to fixed doses of bradykinin (or angiotensin II) was dependent upon enhanced cyclooxygenase synthesis. In order to evaluate changes in renal cyclooxygenase activity we incubated cortical and medullary microsomes (i.e. 100,000 \(\times g\) pellet) produced from rabbit kidneys perfused for 1 to 5 h with \([^{4}C]\)arachidonate in the presence of cofactors. Surprisingly, there was no (quantitative or qualitative) difference in total cyclooxygenase activity between the 1- or 5-h perfused HNK or CLK medullary o- cortical microsomes (Fig. 2). The total cyclooxygenase activity was substantially higher in the medulla than in the cortex of the HNK or the CLK. The apparent site of the presumed in vivo cyclooxygenase induction in the hydronephrotic kidney was in the cortex, whereas, ureter obstruction does not appear to influence medullary arachidonate metabolism (Fig. 2). However, the cortical total cyclooxygenase activity in the HNK microsomes did not increase during the perfusion experiment.
Since the total renal cyclooxygenase activity in hydronephrotic microsomes (from the intact kidney studies with peptide stimulation), did not increase progressively during perfusion as anticipated we decided to evaluate the effect of the cycloheximide and the aspirin treatment on microsomal arachidonate metabolism. The total cyclooxygenase activity of the microsomes was measured by radiochromatography following incubation with \([^14C]arachidonate\) in the presence of cofactors (Table I). Microsomes from CLK or normal rabbit kidneys metabolize less than 1% of arachidonate (not shown), but the hydronephrotic cortex converts 9% of the fatty acid to PGs. Continuous infusion of cycloheximide (for the 5-h perfusion experiment), which caused a 90% reduction in PG release from the bradykinin-stimulated intact perfused kidney (Fig. 1), did not effect the total cortical (or medullar) microsomal cyclooxygenase activity (Fig. 3, Table I). Furthermore, cortical microsomes prepared from aspirin-treated HNK which were nearly fully active (at 5 h of perfusion) in releasing PG following bradykinin stimulation (Fig. 1), were completely inactive in metabolizing the labeled arachidonate (Table I, Fig. 3). In a mixing experiment medullary or cortical microsomes prepared (after 5-h perfusion) from aspirin-treated (in \textit{vivo}) HNK did not inhibit microsomes from a control (untreated) HNK, therefore, precluding the possibility of residual aspirin being released during homogenization.

**PG Synthesis by Endogenous versus Exogenous Arachidonate in Intact Perfused Kidneys—**PG synthesis induced by bradykinin stimulation of the intact kidney necessitates the release of endogenous arachidonate from phospholipids for conversion by renal cyclooxygenase. In microsomal experiments the peptide receptor is lost and the acyl hydrolyase is bypassed in enzymatic incubations that employ \([^14C]arachidonate\) as substrate. We tested the time dependency and the influence of cycloheximide or aspirin on the response of the intact perfused HNK to exogenous arachidonate. The control HNK released progressively more PGE$_2$-like and thromboxane-like contractile activity with time, whereas there was little apparent change in PG production from exogenous arachidonate (determined by bioassay but not shown). Cycloheximide treatment blocked the time-dependent increase in bradykinin induced PG release from the perfused kidney without altering the arachidonate response whereas, the aspirin treatment (in \textit{vivo}) markedly decreased the arachidonate-induced PG production while modestly interfering with the bradykinin response. Quantitation of the venous extract demonstrates there was only a modest change (~2-fold) in total PG production in response to exogenous arachidonate over 4 h (Fig. 4), whereas bradykinin caused a 20-fold increase in PG release over the same time period (Fig. 1). Furthermore, the PG released by bradykinin from endogenous arachidonate was about 4 times greater than that produced by the infusion of the high concentration of exogenous arachidonate (Fig. 1 versus Fig. 4). Aspirin pretreatment resulted in a 90% inhibition (at 5 h of perfusion) of exogenous arachidonate metabolism by the intact perfused HNK (Fig. 4), whereas endogenous arachidonate released by bradykinin was inhibited less than 30% at 5 h (Fig. 1). Cycloheximide did not inhibit exogenous arachidonate metabolism (Fig. 4), whereas, the bradykinin-induced response was inhibited 90% (Fig. 1). Thus, the results obtained with exogenous arachidonate in the intact kidney favorably agree with those obtained by measuring total cyclooxygenase activity (\textit{i.e.} microsomal \([^14C]arachidonate\) conversion) in the renal homogenates.

**Radiochemical Comparison of PG Produced by Exogenous and Endogenous Arachidonate—**The above experiments indicate a marked difference in renal enzymatic conversion of exogenous or endogenous arachidonate in the HNK. These differences are further accentuated by the observation that

**Table I**

<table>
<thead>
<tr>
<th>HNK</th>
<th>PGF$_2 \alpha$</th>
<th>PGE$_2$</th>
<th>PGD$_2$</th>
<th>AA$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortex Control</td>
<td>8,020 ± 2,082</td>
<td>2,906 ± 853</td>
<td>1,944 ± 575</td>
<td>137,801 ± 7,845</td>
</tr>
<tr>
<td>Cycloheximide</td>
<td>9,482 ± 2,050</td>
<td>3,073 ± 915</td>
<td>3,052 ± 937</td>
<td>142,907 ± 2,871</td>
</tr>
<tr>
<td>Aspirin</td>
<td>271 ± 67</td>
<td>178 ± 29</td>
<td>379 ± 88</td>
<td>179,024 ± 3,883</td>
</tr>
<tr>
<td>Medulla Control</td>
<td>46,277 ± 3,303</td>
<td>18,768 ± 2,270</td>
<td>11,940 ± 2,189</td>
<td>70,658 ± 2,519</td>
</tr>
<tr>
<td>Cycloheximide</td>
<td>44,029 ± 3,215</td>
<td>19,202 ± 1,915</td>
<td>15,104 ± 1,290</td>
<td>71,299 ± 7,069</td>
</tr>
<tr>
<td>Aspirin</td>
<td>470 ± 71</td>
<td>236 ± 24</td>
<td>270 ± 37</td>
<td>182,361 ± 2,646</td>
</tr>
</tbody>
</table>

$^a$ AA = arachidonic acid.
different end product PGs are generated by exogenous or endogenous arachidonate. Infusion of $[^{14}C]$arachidonate into the intact perfused hydronephrotic kidney labels the renal phospholipids and simultaneously results in the appearance of $[^{14}C]$-labeled 6-keto-PGF$_{1\alpha}$ in the renal venous effluent (Fig. 5). In contrast, endogenous arachidonate released by bradykinin stimulation of the prelabeled kidneys results in the conversion of the $[^{14}C]$arachidonate (tested at 0, 2, and 4 h) to PG during the loading infusion, but does not inhibit (at 5 h) the bradykinin-induced PGE$_2$ biosynthesis (Fig. 5). The data at 0, 2, and 4 h was the same as at 30 min as shown in Fig. 5.

**FIG. 4.** The conversion of exogenous arachidonate (total cyclooxygenase activity) by the intact perfused hydronephrotic kidneys. The arachidonic acid (AA) was infused for 5 min directly into the renal artery (final arachidonic acid concentration was 1 µg/ml). The values represent the extractable, bioassayable, PG-like contractile activity and are expressed as the means of experiments, the standard errors were ±15%. The cycloheximide curve was not significantly different from the control curve. ASA, acetylsalicylic acid.

**FIG. 5.** Radiochromatographic scan of the acid-lipid extract of the renal venous effluent (50 ml) during $[^{14}C]$arachidonate loading and bradykinin stimulation (1 µg at 6 h) of the hydronephrotic kidney. The standard abbreviations appear in the legend for Fig. 3.

**TABLE II**

<table>
<thead>
<tr>
<th>Experimental conditions</th>
<th>Hydrogenic</th>
<th>Contralateral</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptide-stimulated PLS release by intact perfused kidney</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Large increase proportional to perfusion time</td>
<td>Modest response time-independent</td>
</tr>
<tr>
<td>Cycloheximide</td>
<td>Increase is inhibited</td>
<td>Fully active</td>
</tr>
<tr>
<td>Aspirin</td>
<td>Time-dependent increase following initial delay</td>
<td>Completely inhibited</td>
</tr>
<tr>
<td>AA</td>
<td>No inhibition</td>
<td></td>
</tr>
</tbody>
</table>

*The abbreviations used are: PLS, prostaglandin-like substance; AA, arachidonic acid.*
Regulation of Renal Prostaglandin Synthesis

Our experiments rather strikingly demonstrate major differences in the renal metabolism of exogenous or endogenous (released by bradykinin or angiotensin) arachidonate. The exogenous fatty acid is converted to prostacyclin whereas endogenous arachidonate (released by bradykinin) is predominantly converted to PGE2 (Fig. 5). In addition, ureter obstruction can induce thromboxane synthesis (2, 3). There appears to be a differential distribution of synthetic enzymes in the kidney such that the cyclooxygenase is not coupled to the same endoperoxide-dependent enzyme. One possibility is that preferential renal prostacyclin synthetase (\(\text{i.e., PGI}_2 \rightarrow \text{PGI}_3\)) is only localized in the renal blood vessels. Indeed, \(\text{PGI}_2\) synthesis has been demonstrated in every blood vessel tested (14-16). The lack of recovery of \(\text{PGI}_2\) production from exogenous arachidonate by the perfused aspirin-treated (in vivo) HNK suggests that the newly synthesized cyclooxygenase is not localized in the vasculature but in some other renal site not normally accessible to exogenous arachidonate. The total amount of PG synthesized from exogenous \([^{14}C]\)arachidonate by medullary microsomes was substantially greater (50% conversion) than that in the cortex, but there was no qualitative or quantitative difference between HNK and CLK medullary PG synthesis. On the other hand, in HNK cortical microsomes arachidonate metabolism is facilitated markedly compared to CLK cortical microsomes (Table I). This apparent in vivo change in hydroxynicotinic cyclooxygenase activity is supported by morphological studies which demonstrate enhanced cyclooxygenase-positive immunofluorescence in cortical segments of the collecting tubules (but not in the vasculature) as well as in the thin limb of Henle's loop. The immunofluorescent staining of the vascular cyclooxygenase was quantitatively similar in both the CLK and the HNK. In contrast, normal rabbit kidney cyclooxygenase is localized immunochemically in the endothelium of all arteries and arterioles and in cortical collecting tubules but not in Henle's loop (17).

The absolute site and mechanism of induction of the synthesis of the peptide dependent cyclooxygenase is unresolved. No further change in cyclooxygenase antibody binding was apparent during the 5 h of perfusion of the HNK consistent with our inability to demonstrate a progressive change in total microsomal cyclooxygenase activity.

Cyclooxygenase inhibition of peptide induced PG synthesis in the HNK does not eliminate the possibility that there is \(\text{de novo}\) synthesis of either additional peptide receptor sites or acyl hydrolyase. The aspirin data suggest that new cyclooxygenase is being synthesized during the perfusion period but that no data on the status of the lipase or the receptor. Morrison et al\(^3\) have evidence that suggests a marked increase in peptide-stimulated (but not basal) lipase activity in the intact perfused HNK following 5 h of perfusion. Thus, the activation of new protein synthesis during perfusion of ureter-

\(^3\)W. Smith and P. Needleman, submitted for publication.

\(^3\)A. R. Morrison, N. Pascoe, and P. Needleman, submitted for publication.

FIG. 6. Schematic diagram illustrating the peptide-linked stimulation of cyclooxygenase. Exogenous arachidonic acid (AA) bypasses the requirement for a cell membrane receptor or for activation of an acyl hydrolyase and is directly converted by cyclooxygenase. This cyclooxygenase does not appear inducible (irreversibly blocked by aspirin) and is not sensitive to cycloheximide. Peptide (BK, bradykinin; \(\text{ANG II, angiotensin II}\)) stimulation must activate a receptor and specific phospholipase (PLA2) to provide arachidonate for the specific cyclooxygenase. It is this latter complex that is (a) inducible in hydronephrosis; (b) inhibitible by cycloheximide; and (c) recovers after aspirin inactivation.
obstructed rabbit kidneys may well involve the entire enzyme complex of phospholipase, cyclooxygenase, and thromboxane synthetase. This complex of enzymes appear to be tightly coupled (i.e. highly efficient conversion of specifically released arachidonate), highly selective for peptide activation, and readily vulnerable to regulation in pathophysiological states.

REFERENCES
Specific regulation of peptide-induced renal prostaglandin synthesis.

P Needleman, A Wyche, S D Bronson, S Holmberg and A R Morrison