Influence of Renal Prostaglandin Synthesis on Renin Control Mechanisms in the Dog

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SUMMARY We studied the influence of altered rates of intrarenal prostaglandin synthesis on known renin control mechanisms in single, filtering and nonfiltering, denervated kidneys of chloralose-anesthetized dogs. Infusion of indomethacin directly into the renal artery resulted in 80% reductions in both renin and prostaglandins effluxing from the renal vein. The increased plasma renin resulting from supraprenal aortic constriction was unaffected by indomethacin despite suppressed renal vein prostaglandin efflux. However, the renin response to furosemide was blunted by prior renal arterial indomethacin administration even though furosemide had no influence on prostaglandin efflux. Renin secretion also was suppressed by intrarenal indomethacin administration in nonfiltering kidneys. Intrarenal arachidonic acid infusion resulted in parallel increases in renin and prostaglandin in both papaverine-treated and untreated, nonfiltering, denervated kidneys. Imposition of intrarenal indomethacin during arachidonic acid infusion in nonfiltering kidneys suppressed prostaglandin efflux from both papaverine-treated and untreated kidneys; however, renin secretion was suppressed by indomethacin in only the control kidney. Since renin was stimulated by arachidonic acid in nonfiltering, denervated, papaverine-treated kidneys, it is concluded that the in vivo intrarenal cyclooxygenase-dependent conversion of arachidonic acid to prostaglandins exerts its influence on renin secretion by a direct influence on juxtaglomerular cells and is independent of other presently recognized renin control mechanisms. Circ Res 45: 13-25, 1979

CONSIDERABLE interest has developed over the past several years concerning mechanisms whereby intrarenal prostaglandins (PG) or their intermediate synthetic products may influence renin secretion. This interest has sprung from the observation that indomethacin, an inhibitor of the renal cyclooxygenase-dependent conversion of arachidonic acid to prostaglandins, suppresses renin in a variety of species including man (Flower, 1974; Larsson, et al., 1974; Romero and Strong, 1977; Yun et al., 1977; Frolich et al., 1976a; Romero et al., 1976). In addition, the rate at which renin is released from renal cortical slices is enhanced by arachidonic acid and by both natural and stable synthetic PG endoperoxides (Weber et al., 1976) and by prostacyclin, PG12 (Gerber et al., 1978). Although such evidence suggests that PG synthetic events may modify renin secretory mechanisms, little is known of how this may be accomplished in vivo. There appear to be several potential pathways whereby intrarenal PG synthetic events may influence renin control mechanisms. Intrarenal PG synthesis might affect the renal baroreceptor by modifying PG vasoactive influences. It could modify renal tubular electrolyte handling and thus affect changes on macula densa-dependent mechanisms, and, finally, intrarenal

PG's might modify sympathetic nervous input to renin secretion. All of these are known to regulate renin release (Davis and Freeman, 1976). Indomethacin administration has been reported to reduce the renin response to hemorrhage (Romero et al., 1976) and furosemide (Frolich et al., 1976a) but to be ineffective in preventing the renin response to isoproterenol (Frolich et al., 1976b). Studies such as these suggest that the in vivo interaction of PG's with renin may be via a modification of the presently recognized renin control mechanisms.

Since the in vivo link between intrarenal PG's and renin secretory mechanisms is as yet undefined, we undertook a systematic series of studies to examine the influence of direct intrarenal indomethacin administration on maneuvers that stimulate renin primarily through intrarenal mechanisms. Taken as a whole, these data appear to indicate that the in vivo interaction of PG synthesis and renin secretion is through a direct effect on juxtaglomerular cells rather than secondary to modification of primary renin control mechanisms.

Methods

All studies were conducted in the sole remaining denervated kidney of anesthetized mongrel dogs. This experimental model eliminated neural input and removed humoral contributions of an intact contralateral kidney. We studied the effect of infusing indomethacin directly into the renal artery on circulating basal renin and on renin stimulated by reduced renal perfusion pressure, by furosemide administration, and by isoproterenol infusion in separate groups of dogs with filtering kidneys. PG's
effluxing from the renal vein were measured throughout as an index of intrarenal PG synthetic status. In a final series, renin secretion was studied during enhanced and suppressed rates of PG synthesis in single, papaverine-treated and untreated (control) denervated, nonfiltering kidneys.

**General Surgery**

Adult mongrel dogs of both sexes were anesthetized with sodium pentobarbital (30 mg/kg, iv). Both kidneys were exposed via retroperitoneal flank incisions under sterile techniques. The left renal artery, renal vein, and ureter were stripped of all visible nerves and then painted with a solution of 10% phenol in absolute ethanol to destroy any nerves concealed within the adventitia. Occasionally, the left kidney was supplied by more than one renal artery. In those instances the right kidney was used for study. The contralateral kidney then was excised. Two to 3 days after these preparatory procedures, the dogs were anesthetized with sodium methohexital (10 mg/kg, iv) and maintained with a solution of 1% α-chloralose. A catheter was positioned in the suprarenal aorta via the right femoral artery. This aortic catheter was used to obtain arterial blood samples and to monitor arterial blood pressure (Statham 23Db strain gauge). A second polyethylene catheter was inserted into the vena cava through the right femoral vein to provide a route for drug administration and fluid replacement. The trachea was intubated with auffed endotracheal tube and positive pressure ventilation initiated with a Harvard Instruments model 607 respiratory pump. Respiratory CO₂ was monitored continuously with a Beckman LB-2 respiratory gas analyzer. Tidal volume was adjusted to maintain end-expiratory gases at approximately 4% CO₂. This precaution was observed to assist in maintaining acid-base status during the experimental procedures.

The dogs were suspended in an upright position and the denervated kidney was exposed. Curved 22-gauge needles, attached to thin-wall Silastic tubing, were placed in the renal vein and artery for sampling of renal venous blood and for direct intrarenal arterial infusions. A noncannulating electromagnetic blood flow probe, interfaced with a Zepeda Instruments model SW-3 square-wave flowmeter, was positioned around the renal artery for renal blood flow (RBF) measurements. The ureter was ligated and a polyethylene catheter was inserted for timed urine collections. A 1% NaHCO₃ solution was infused intravenously at a rate such that urine flow was maintained at approximately 1 ml/min. Glomerular filtration rate (GFR) was determined by the clearance of radioactive iothalamate, a method comparable to the clearance of inulin (Sigman et al., 1965). At least 40 minutes prior to sampling, a 0.5 μCi/kg bolus of 125I-iothalamate (Glofil-125, Abbott Labs) was injected intravenously and followed by a sustaining infusion (1000 counts/min). Each clearance period consisted of a 20-minute urine collection with an arterial plasma sample drawn at the midpoint. Urine and plasma iothalamate concentrations were proportional to counts/min per ml, which were measured with a Nuclear-Chicago gamma counter. The iothalamate clearance (assumed to equal GFR) was calculated as 

\[
\frac{[U]}{[P]}
\]

where [U] and [P] are counts/min per ml of urine and plasma, respectively, and \( \dot{V} \) is urine flow rate in ml/min.

**Measurements**

In all procedures, pulsatile and mean arterial blood pressure and RBF were recorded continuously on a Beckman type RM 6-channel dynograph. Cardiovascular variables were analyzed and blood samples were drawn for renin and PG during the final minute of each period. Blood and urine samples were collected for measurement of the following parameters: arterial plasma renin activity (PRA), renal venous PG effluent, GFR, serum Na⁺ and K⁺ concentrations, and Na⁺ and K⁺ excretions. Renin activity and secretion were determined by methods previously described (Zehr et al., 1976) adapted from those of Haber et al. (1969). Briefly, 2 ml of blood were collected in plastic syringes containing 0.1 ml of 10% sodium ethylenediaminetetraacetate, immediately transferred to iced glass tubes, and chilled to 0°C. Hematocrits were determined by microhematocrit methods. Converting enzyme and angiotensinase activities were suppressed by the addition of 0.02 ml of 5% diisopropyl fluorophosphate to 1 ml of the separated plasma. Neomycin sulfate (0.02 ml) was added as a bacterial retardant. To ensure that the renin-substrate reaction was not substrate limiting, an aliquot of partially purified homologous renin substrate, extracted from the plasma of 48-hour nephrectomized dogs (Haas et al., 1966), was added to each sample. Samples were acidified to pH 5.8 with 1 N HCl prior to generation of angiotensin (2 hours at 39°C). A sample was removed prior to generation for blank determinations. All assays were conducted in triplicate, and all samples for a given dog were assayed at the same time against a common standard curve. If reassay at a different dilution was required, the entire experimental run was reassayed at the new dilution. Renin activity is expressed as nanograms of angiotensin I formed per milliliter of plasma per hour of generation (ng/ml per hr), and renin secretion was calculated as the product of renal venous minus arterial renin activities multiplied by renal plasma flow [RBF(1-Hct) \( \times \) (V–A) renin activity] and is expressed as ng/min.

Renal venous immunoreactive PG (i-PG) was measured using a radioimmunoassay kit prepared by Clinical Laboratories. Neutral lipids first were extracted from renal venous blood with petroleum ether. Prostaglandins then were extracted from the aqueous phase with a mixture of ethyl acetate-isopropyl alcohol-0.2 N HCl (3:3:1). After the addition of 2 ml of ethyl acetate and 3 ml of water, the PG-
containing organic phase was transferred to polypropylene tubes and dried at 55°C under an air stream. The residue was taken up in isogel-Tris buffer, alkalized to pH 12.5, and heated in a boiling water bath for 5 minutes. The pH of the solution then was returned to 7.4. This procedure converts PGE's (and A's if present) to PGB. Immunossay of the mixture with an antibody specific for PGB yields an estimate of PG concentration. Tracer amounts of \(^{3}H\)-PGE were added to each plasma sample prior to initiation of the procedure for purposes of efficiency correction.

Although PGE\(_2\) and PGF\(_\alpha\) have been recognized as major components of cyclooxygenase-dependent metabolism of arachidonic acid in the kidney, it has become increasingly apparent that other biologically active intermediates and end products are also present. In addition to E\(_2\), both the cyclic endoperoxides (Weber et al., 1976) and prostacyclin-PGI\(_2\) (Gerber et al., 1978) have been shown to enhance renin release. Since renal synthesis of the cyclic endoperoxides, prostacyclin, and PG end products is cyclooxygenase dependent, the assay of PG-like compounds in the present study is considered to be only a qualitative index of the state of cyclooxygenase activity. Thus the term "immunoreactive-PG" (i-PG) will be used throughout, and no implications regarding the identity of the various biologically active species are intended. The total renal venous effluent (ng/min) was derived from the assayed renal plasma i-PG concentration and the renal plasma flow [(1-hematocrit) \(\times\) RBF]. This value was considered to reflect most accurately intrarenal i-PG synthesis during the experimental manipulations, some of which altered RBF. Serum and urinary Na\(^+\) and K\(^+\) concentrations were determined with an Instrument Laboratories flame photometer. Urinary excretion rates (mEq/min) were calculated by multiplying ion urinary concentration by the urine flow rate.

**Statistical Analysis**

Analysis of variance was applied to those data comprised of multiple times or treatment groups. In the event the F ratio (treatment mean square/error mean square) indicated that changes had occurred, a Tukey comparison of means test was used to identify individual differences (Li, 1964). Whenever appropriate, differences between responses of the control dogs and those treated with indomethacin were analyzed by the Student's comparison of means t-test. Minimum criteria for rejection of the null hypothesis were set at the 95% confidence level.

**Experimental Protocol**

Following the completion of experimental surgical procedures, a 60-minute equilibration period was allowed. Two control clearance periods were observed and the experimental perturbation was then imposed. The following series of experiments were conducted.

**Series 1: Renin and i-PG Responses to Intrarenal Indomethacin Infusion**

The purpose of studies in this group was to establish the concomitant responses of PRA and i-PG during cyclooxygenase blockade. The effects of an intrarenal indomethacin infusion (0.1 mg/kg per min) were examined in six dogs that had undergone unilateral nephrectomy and renal denervation. Saline (1 ml/min) was infused into the renal artery throughout the surgical recovery and the two control periods with a Harvard Apparatus infusion-withdrawal syringe pump. Immediately following the control clearances, the saline was replaced by indomethacin (Merck, Sharp, and Dohme) dissolved in 1% NaHCO\(_3\). The drug infusion was maintained at 0.1 mg/kg per min for 60 minutes at a volume of 1 ml/min.

**Series 2: Effect of Intrarenal Indomethacin on Renin and i-PG Responses to Reduced Renal Perfusion Pressure**

The purpose of studies in this group was to test the hypothesis that intrarenal PG's might influence renal baroreceptor release of renin. In addition to the general surgical procedures, a Blalock clamp was placed around the suprarenal aorta in 11 dogs. A second aortic catheter, positioned near the origin of the renal artery, was used for renal perfusion pressure measurements. Five of the dogs received indomethacin (0.1 mg/kg per min) into the renal artery for 40 minutes prior to the experimental maneuver. The responses to a 20-minute constriction of the aorta were compared in control dogs (\(n = 6\)) with those in the indomethacin-blocked group subjected to a similar constriction.

**Series 3: Effect of Intrarenal Indomethacin on Renin and i-PG Responses to Furosemide Administration**

The goal of studies in this group was to test whether intrarenal PG's modify renal tubular dependent release of renin. In 12 dogs, an additional catheter was inserted into the left femoral vein. Saline (0.9% NaCl) was infused (0.5 ml/min) through this venous catheter throughout the equilibration and control periods. Immediately before a final 20-minute clearance, a 5 mg/kg bolus of furosemide (Lasix) was injected and the intravenous saline infusion replaced with furosemide (1 mg/kg per hr). The furosemide-induced renin responses were compared in control dogs (\(n = 6\)) and in dogs (\(n = 6\)) that had received intrarenal indomethacin (0.1 mg/kg per min) for 40 minutes prior to and during furosemide administration.

**Series 4: Effect of Intrarenal Indomethacin on Renin and i-PG Response to Intravenous Isoproterenol**

We studied this group to determine whether intrarenal PG's modify \(\beta\)-adrenergic-mediated release of renin. Saline (0.5 ml/min) was infused via a
femoral venous catheter in 17 dogs during the surgical equilibration and control periods as before. During a final 20-minute clearance, intravenous isoproterenol infusion (0.02 μg/kg per min) was substituted for the saline and the results were compared between control dogs (n = 9) and those (n = 8) receiving intrarenal indomethacin (0.1 mg/kg per min) for 40 minutes prior to and during isoproterenol administration.

Series 5: Effect of Enhanced and Suppressed PG Synthesis on Renin Secretion in Nonfiltering Kidneys

We designed this series to determine whether PG synthetic events have a direct in vivo influence on renin secretion independent from the major control factors previously discussed. Twenty dogs were subjected to the surgical procedures developed by Blaine et al. (1970) for producing nonfiltering kidneys. Briefly, the dogs were anesthetized with pentobarbital (30 mg/kg, iv) and an aseptic flank exposure of the left kidney was performed. The kidney was first denervated, as described above, and an atraumatic arterial bulldog clamp was used to produce a 2-hour period of renal ischemia. The ureter was then ligated and cut. Following surgical closure, the dogs recovered uneventfully until the day of the experiment. Four to 5 days after this procedure, the dogs were anesthetized and prepared as described for the previous groups. The contralateral filtering kidney then was removed via a retroperitoneal flank incision. In addition, curved 22-gauge needles attached to thin-wall silicone rubber tubing were inserted into the renal artery and vein of the sole nonfiltering kidney for infusion and blood sampling. Renin secretion rates were determined from the product of renal plasma flow [(1-Hct) x RBF] and venous-to-arterial renin activity gradient. In addition to i-PG efflux and renin secretion, renal efflux of cyclic adenosine 3',5'-monophosphate (cAMP) was determined. Plasma proteins first were precipitated with 0.6 N perchloric acid. The nucleotide was layered onto Dowex 50 W-X8 (100-200 mesh) resin in the H+ form and eluted with distilled water. After the first 3 ml of the eluent were discarded, the next 5 ml were collected and dried in an air stream at 60-70°C. The residue was dissolved in acetate buffer, and cAMP was determined by radioimmunoassay with a kit prepared by Schwartz/Mann. The rate of renal venous effluent was calculated by multiplying the renal plasma flow [(1-Hct) x RBF] by the assayed cAMP concentration. Values are expressed as pmol/min. After completion of each experimental procedure, two independent methods were employed to verify that the kidney was, indeed, nonfiltering. The ligated ureter was opened and a 40-minute GFR was determined by clearance of 125I-iothalamate (Abbott Laboratories), as described above. A portion of the renal capsule then was removed, and surface nephrons were observed under a dissecting microscope during intra-arterial injection of lissamine green, a dye which is readily filtered. Dogs were rejected if 125I-iothalamate clearance exceeded 5 ml/min or if lissamine green appeared in the surface tubules. Mean glomerular filtration in the dogs accepted was calculated to be approximately 1.0 ml/min, and we assume tubular function was insignificant. In each protocol a 60-minute equilibration period was observed. One of the following three experimental protocols then was conducted.

Protocol A: Effect of Intrarenal Indomethacin

Following collection of control data, indomethacin (0.1 mg/kg per min) was infused into the renal artery for a 60-minute period as in the previous groups. Renin secretion rates were determined at 20-minute intervals and correlated with recorded cardiovascular variables at those time points. These data were gathered in five dogs to determine the basic nonfiltering kidney response to indomethacin. Since it was clear that renin was suppressed by this procedure, and since the use of the nonfiltering denervated kidney restricted possible renin responses to either baroreceptor or a direct juxtaglomerular action, we undertook the following two additional studies to differentiate these possibilities.

Protocol B: The Effect of Arachidonic Acid and Arachidonic Acid Combined with Indomethacin in Nonfiltering Kidneys. In this group of six dogs, control data were determined as described above. Arachidonic acid, the primary renal PG precursor, then was infused directly into the renal artery of the sole nonfiltering kidney at a rate of 15 μg/kg per min (Tannenbaum et al., 1975) throughout the remaining 40 minutes of the experiment. After 20 minutes of arachidonic acid infusion, all data were gathered as before. During the final 20 minutes, indomethacin (0.1 mg/kg per min) was infused through a second renal arterial needle catheter. With this protocol it was possible to document renin and i-PG secretion responses during both enhanced and suppressed PG-cyclooxygenase activity in the presence of elevated but constant delivery of substrate for PG synthesis. Arachidonic acid (5,8,11,14-eicosatetraenoic acid) was obtained (Sigma) in sealed nitrogen ampules and stored at -20°C. Immediately before each experiment, a stock solution of 10 mg arachidonic acid per ml in absolute ethanol was prepared in a light-protected, nitrogen atmosphere. For the intrarenal infusion of 15 μg/kg per min, an appropriate volume of the ethanol stock solution was diluted with saline. At all times, precautions were taken to minimize arachidonic acid oxidation by protecting the infusate from light and air.

Protocol C: The Effect of Arachidonic Acid and Arachidonic Acid Combined with Indomethacin in Papaverine-treated, Nonfiltering Kidneys. The protocol of this group of nine dogs was identical to
Table 1 Cardiovascular and Renal Responses to Intrarenal Indomethacin Infusion (0.1 mg/kg per min) into Denervated Kidneys of Unilaterally Nephrectomized Dogs

<table>
<thead>
<tr>
<th></th>
<th>Average control</th>
<th>Indomethacin (0.1 mg/kg per min)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>20 min</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>135.3 ± 5.3</td>
<td>138.3 ± 6.8</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>84 ± 10</td>
<td>73 ± 10</td>
</tr>
<tr>
<td>RBF (ml/min)</td>
<td>132.5 ± 13.8</td>
<td>121.0 ± 12.2</td>
</tr>
<tr>
<td>GFR (ml/min per g)</td>
<td>0.706 ± 0.106</td>
<td>0.725 ± 0.119</td>
</tr>
<tr>
<td>Na+ excretion (mEq/min)</td>
<td>0.103 ± 0.013</td>
<td>0.207 ± 0.073†</td>
</tr>
<tr>
<td>K+ excretion (mEq/min)</td>
<td>0.036 ± 0.008</td>
<td>0.054 ± 0.016</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SE. A time-variant analysis combined with a Tukey comparison of individual means was applied to test the null hypothesis that changes had occurred. *P values indicate the confidence level that a given experimental point was different from control; n = 6.

During suprarenal aortic constriction, renal perfusion pressure and blood flow decreased to similar levels in control and indomethacin-blocked dogs (Table 2). Mean pressure proximal to the clamp increased to similar values during the experimental period in both groups.

Total renal venous i-PG effluent in control dogs was unchanged after 20 minutes of aortic constriction. Baseline PG effluent from the indomethacin-treated kidney (P < 0.01) was lower than that from the control group and decreased further during the clamping episode (Table 2). Partial renal ischemia increased PRA from initial levels of 3.5 ± 1.7 to 13.8 ± 4.5 in untreated dogs and from 3.9 ± 1.7 to 13.8 ± 4.4 in indomethacin-blocked dogs. The response was similar in both groups (Fig. 2).
### Table 2: Cardiovascular Responses to Reduction of Renal Perfusion Pressure in Control and in Indomethacin-Blocked Dogs with a Single Denervated Kidney

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 6)</th>
<th>Indomethacin (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Clamp</td>
</tr>
<tr>
<td>Renal perfusion pressure (mm Hg)</td>
<td>115.8 ± 7.5</td>
<td>58.6 ± 2.3*</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>118.0 ± 7.5</td>
<td>135.3 ± 12.0*</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>101 ± 13</td>
<td>91 ± 12</td>
</tr>
<tr>
<td>RBF (ml/min)</td>
<td>178.4 ± 33.9</td>
<td>88.3 ± 16.0*</td>
</tr>
<tr>
<td>GFR (ml/min per g)</td>
<td>0.684 ± 0.079</td>
<td>0.004 ± 0.002*</td>
</tr>
<tr>
<td>Na⁺ excretion (mEq/min)</td>
<td>0.181 ± 0.048</td>
<td>0.002 ± 0.001*</td>
</tr>
<tr>
<td>i-PG effluent (ng/min)</td>
<td>434 ± 139</td>
<td>507 ± 192</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SE. Student's paired t-test was applied, both in the presence and absence of intrarenal indomethacin infusion, to test the null hypothesis that values observed during control were equal to those observed during reduced renal perfusion pressure.

* P < 0.01; † P < 0.025; ‡ data not available.

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**Series 3: Effect of Intrarenal Indomethacin on Renin and PG Responses to Furosemide Administration**

In this group, the responses to furosemide were studied in control dogs and in those receiving indomethacin directly into the renal artery for 40 minutes prior to and during the administration of the diuretic.

Cardiovascular, renal, and i-PG responses to furosemide administration are presented in Table 3. Mean pressure and RBF in control and in indomethacin-blocked dogs were not significantly altered by furosemide treatment. GFR fell after 20 minutes of furosemide administration in both groups, although the decrease was statistically significant only in the indomethacin-infused dogs. Sodium excretion significantly increased with the diuretic treatment in controls and in PG-inhibited animals, whereas K⁺ excretion was unchanged. Serum Na⁺ and K⁺ concentrations were within the normal range for dogs and did not significantly change in any of them.

Renal venous i-PG effluent was not affected by furosemide treatment in either group (Table 3). On the other hand, furosemide administration resulted in increases in PRA from initial levels of 3.2 ± 0.8 to 18.3 ± 5.1 ng/ml per hr in untreated dogs, whereas increases from 1.2 ± 0.3 to 6.1 ± 1.7 were observed in the indomethacin-blocked group (Fig. 3). The actual response during furosemide was significantly greater in the untreated group (P < 0.02).

**Figure 2:** The effect of intrarenal indomethacin infusion on the renin response to a controlled reduction in renal perfusion pressure in dogs with a single filtering, denervated kidney. Clamp values were obtained 20 minutes after reduction of renal perfusion pressure by 50%. Student's paired t-test was used to compare renin activity during renal ischemia with its respective control value (*P < 0.05).

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**Series 4: Effect of Intrarenal Indomethacin on Renin and PG Responses to Intravenous Isoproterenol**

In this group, the responses to β-adrenergic activation were studied in control dogs and in dogs with intrarenal indomethacin infusion for 40 minutes prior to administration of isoproterenol.

Twenty minutes of intravenous isoproterenol infusion significantly decreased mean arterial pressure, RBF, and GFR, and increased heart rate in both control and indomethacin-treated dogs (Table 4). In both groups, sodium excretion was unchanged whereas K⁺ excretion was reduced significantly during isoproterenol administration.

During the control period, renal venous i-PG efflux from indomethacin-blocked kidneys was significantly less (P < 0.01) than that from the control kidneys. No significant i-PG response to isoproterenol.
Table 3 Cardiovascular and Renal Responses to Furosemide in Control and in Indomethacin-Blocked Dogs with a Single Denervated Kidney

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 6)</th>
<th>Indomethacin (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average control</td>
<td>Furosemide</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>121.8 ± 6.0</td>
<td>118.8 ± 9.0</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>83 ± 5</td>
<td>93 ± 5*</td>
</tr>
<tr>
<td>RBF (ml/min)</td>
<td>193.8 ± 10.5</td>
<td>198.2 ± 26.2</td>
</tr>
<tr>
<td>GFR (ml/min per g)</td>
<td>0.737 ± 0.097</td>
<td>0.549 ± 0.160</td>
</tr>
<tr>
<td>Na⁺ excretion (mEq/min)</td>
<td>0.312 ± 0.081</td>
<td>0.719 ± 0.175†</td>
</tr>
<tr>
<td>K⁺ excretion (mEq/min)</td>
<td>0.134 ± 0.093</td>
<td>0.222 ± 0.167</td>
</tr>
<tr>
<td>i-PG effluent (ng/min)</td>
<td>537 ± 279</td>
<td>457 ± 311</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SE. Student’s paired t-test was applied, both in the presence and absence of indomethacin, to test the null hypothesis that values observed during control were equal to those observed during furosemide administration.

* P < 0.05; †P < 0.01.

Isoproterenol was observed in either saline-infused or indomethacin-blocked kidneys (Table 4).

Isoproterenol produced significant increases in PRA (Fig. 4) in control (2.54 ± 0.89 to 8.79 ± 3.40 ng/ml per hr) and in indomethacin-treated dogs (1.11 ± 0.52 to 3.23 ± 0.80 ng/ml per hr). In both instances PRA increased approximately 3-fold from their respective control values. No statistically significant difference exists in either the absolute change in the percent change when the actual responses of the individual groups are compared.

Series 5: Effect of Enhanced and Suppressed PG Synthesis on Renin Secretion in Nonfiltering Kidneys

This series, consisting of three separate protocols, was designed to determine whether intrarenal PG’s exert direct effects on juxtaglomerular cells. The basic renin secretion response to intrarenal indomethacin infusion into nonfiltering kidneys (protocol A) is depicted in Figure 5. By the end of 60 minutes, renin secretion had been reduced from a control value of 83.7 ± 28.2 to 23.9 ± 16.2 ng/min. Cardiovascular data are given in Table 5. The low RBF, characteristic of nonfiltering kidneys, was decreased progressively throughout indomethacin administration.
TABLE 4  Cardiovascular and Renal Responses to Isoproterenol in Control and in Indomethacin-Blocked Dogs with a Single Denervated Filtering Kidney

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 9)</th>
<th>Isoproterenol (n = 9)</th>
<th>Indomethacin (n = 8)</th>
<th>Isoproterenol (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>120.1 ± 7.2</td>
<td>98.7 ± 6.8*</td>
<td>118.4 ± 4.9</td>
<td>94.9 ± 4.9*</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>106.1 ± 8.9</td>
<td>140.3 ± 9.0</td>
<td>79.1 ± 9.7</td>
<td>137.8 ± 13.1*</td>
</tr>
<tr>
<td>RBF (ml/min)</td>
<td>190.4 ± 26.7</td>
<td>163.3 ± 24.3</td>
<td>126.5 ± 19.3</td>
<td>92.4 ± 16.9†</td>
</tr>
<tr>
<td>GFR (ml/min per g)</td>
<td>0.602 ± 0.148</td>
<td>0.472 ± 0.180†</td>
<td>0.919 ± 0.156</td>
<td>0.622 ± 0.171*</td>
</tr>
<tr>
<td>Na⁺ excretion (mEq/min)</td>
<td>0.123 ± 0.041</td>
<td>0.109 ± 0.066</td>
<td>0.066 ± 0.014</td>
<td>0.067 ± 0.022</td>
</tr>
<tr>
<td>K⁺ excretion (mEq/min)</td>
<td>0.031 ± 0.007</td>
<td>0.019 ± 0.007*</td>
<td>0.026 ± 0.009</td>
<td>0.019 ± 0.007*</td>
</tr>
<tr>
<td>i-PG effluent (ng/min)</td>
<td>518 ± 128.2</td>
<td>445 ± 212.1</td>
<td>52 ± 20.8</td>
<td>31 ± 16.9</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SE. Student’s paired t-test was applied, both in the presence and absence of indomethacin, to test the null hypothesis that values observed during control were equal to those observed during isoproterenol infusion.

* P < 0.05, † P < 0.01.

infusion (P < 0.005). cAMP effluxing from the renal vein was also decreased (P < 0.005).

In protocol B, intrarenal PG’s were first increased by AA infusion and then depressed with indomethacin. Cardiovascular, i-PG efflux, and renin secretion data are shown in Table 6 and Figure 6 (note log scale), respectively, for the dogs in this group. Five of the six dogs in this group showed increased renin secretion during administration of arachidonic acid, and decreased secretion when indomethacin was imposed additionally. One of the dogs showed an opposite response and was considered an aberrant subject since it could be excluded on statistical grounds (Li, 1964).

Figure 6 shows that, after 20 minutes of intrarenal arachidonic acid infusion, renin secretion had increased to approximately four times that of control (17.4 ± 3.7 to 68.3 ± 22.5 ng/min). Although mean i-PG efflux had doubled, considerable experimental variation existed. As a result, this response was not statistically significant (P ~ 0.1). After 20
minutes of intrarenal indomethacin infusion, both renin and i-PG secretion rates had returned to near control levels despite the continued delivery of arachidonic acid. With the exception of a bradycardia during indomethacin, no statistically significant changes were observed in other measured variables (Table 6). The observation of a bradycardia was consistent with that seen during earlier groups of dogs.

In a final group of dogs, protocol C, papaverine was infused directly into the renal artery at a dose (4 mg/min) previously shown (Witty et al., 1971) to block effectively the renal baroreceptor. A study identical to that of protocol B was then conducted in these nonfiltering kidneys treated with papaverine. Renin secretion again was increased approximately four times during arachidonic acid infusion (87.6 ± 32.8 to 348 ± 162 ng/min).

However, indomethacin failed to suppress renin secretion even though i-PG efflux was reduced markedly (Fig. 7). Cardiovascular and cAMP data for this group are summarized in Table 7. An interesting observation was that cAMP efflux was elevated when compared with the other groups \((P < 0.05)\), probably a reflection of the fact that papaverine is a cAMP-phosphodiesterase inhibitor. It is

---

**Table 5** Responses to Intrarenal Indomethacin Infusion (0.1 mg/kg per min) into Nonfiltering Kidneys \((n = 5)\)

<table>
<thead>
<tr>
<th></th>
<th>Average control</th>
<th>Indomethacin (0.1 mg/kg per min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 min</td>
<td>40 min</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>95.0 ± 5.8</td>
<td>90.6 ± 7.3</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>119.2 ± 15.7</td>
<td>106.8 ± 11.3</td>
</tr>
<tr>
<td>RBF (ml/min)</td>
<td>54.6 ± 7.2</td>
<td>32.1 ± 3.0*</td>
</tr>
<tr>
<td>Renal venous cAMP efflux (pmol/min)</td>
<td>321.9 ± 77.3</td>
<td>183.0 ± 35.9</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SE.

A time-variant analysis was applied to test the null hypothesis that mean values for a given variable were equal at each time point. A Tukey comparison of means was applied to identify data points deviating from the control value:

\* \(P < 0.005\).

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**Table 6** Responses to Intrarenal Arachidonic Acid (AA) (15 \(\mu g/kg\) per min) and Indomethacin (0.1 mg/kg per min) Infusion into Nonfiltering Kidneys \((n = 5)\)

<table>
<thead>
<tr>
<th></th>
<th>Average control</th>
<th>AA</th>
<th>20 min AA + indomethacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>103.6 ± 12.8</td>
<td>96.0 ± 14.9</td>
<td>97.6 ± 16.1</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>89.8 ± 4.9</td>
<td>88.4 ± 6.8</td>
<td>71.6 ± 3.7*</td>
</tr>
<tr>
<td>RBF (ml/min)</td>
<td>68.5 ± 12.7</td>
<td>91.7 ± 23.7</td>
<td>73.3 ± 21.3</td>
</tr>
<tr>
<td>Renal venous cAMP efflux (pmol/min)</td>
<td>291.4 ± 42.1</td>
<td>354.6 ± 90.6</td>
<td>234.7 ± 56.8</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SE.

Analysis of variance was applied to test the null hypothesis that the treatment mean values for each variable were equal. A Tukey comparison of means was used to identify individual differences:

\* \(P < 0.05\) compared to control and AA.

---

**Figure 7** The effect of intrarenal arachidonic acid (15 \(\mu g/kg\) per min) infusion and superimposed indomethacin infusion (0.1 mg/kg per min) on renin and prostaglandin secretion in dogs with a sole, nonfiltering, denervated, papaverine-treated kidney. Analysis of variance combined with a Tukey comparison of individual means was applied to derive the indicated \(P\) values (\(^*\) \(P < 0.025\); \(^†\) \(P < 0.05\); \(n = 9\)).
apparent that the renal vascular tree was dilated maximally, since renal blood flow in this group was double that seen in the group that received no papaverine throughout control and experimental procedures. The renal baroreceptor was assumed to exert little, if any, influence under these conditions.

Discussion

These experiments demonstrate that infusion of indomethacin (0.1 mg/kg per min) directly into the denervated filtering kidney of anesthetized dogs suppresses basal renal levels and modifies the renin response to furosemide administration. However, renin responses to reduced renal perfusion pressure and isoproterenol administration remained during intrarenal indomethacin. A number of studies have demonstrated previously that indomethacin administration is associated with a decrease in renin. However, the many variables in intact subjects which may alter renin secretion complicate the task of characterizing a precise mechanism for intrarenal renin-PG interaction. No systematic study of the effect of direct intrarenal indomethacin administration during activation of the primary factors controlling renin secretion has been reported previously. The first phase of these studies was conducted in a sole remaining denervated filtering kidney to restrict primarily the renin response to intrarenal mechanisms. Renal denervation eliminated the influences of the renal sympathetic nerves on renin secretion (Johnson et al., 1971), PG output (Dunham and Zimmerman, 1970), and renal vascular resistance (Schrier, 1974). Circulating angiotensin II has also been shown to modify renin release (McDonald et al., 1975) and renal venous PG concentration (McGiff et al., 1970). Renin (consequently angiotensin) contributed by an intact untreated kidney may modify the responses of the indomethacin-infused kidney and complicate interpretation of the data. To minimize these influences the contralateral intact kidney was removed in all the experimental dogs.

Several of the responses to indomethacin infusion into these denervated one-kidney dogs resembled the changes reported in intact animals. It has been shown that RBF is reduced in intact anesthetized dogs treated with indomethacin (2 mg/kg) (Lonigro et al., 1973). PG inhibition in the denervated kidneys in our study also was accompanied by a decrease in RBF. A quantitative comparison of the magnitude of the responses is unwarranted due to a difference in the dose and route of administration of the drug. Despite differences in experimental conditions, GFR was not affected by indomethacin in these denervated one-kidney dogs, in conscious rats (Düsing et al., 1976), or in anesthetized rats (Leyssac et al., 1975).

The systemic cardiovascular observations during i-PG inhibition in the present study differ somewhat from previous reports. The increase in arterial pressure following intravenous indomethacin treatment (Lonigro et al., 1973) was not observed during intrarenal drug infusion in our dogs. Our method of direct renal arterial indomethacin infusion was chosen to maximize the drug's effect on renal cyclooxygenase activity when minimizing as much as possible its systemic effects. Despite this, indomethacin infusion consistently resulted in a significant bradycardia within 40 minutes. The mechanism by which the pulse was slowed by indomethacin treatment cannot be deduced from the present data.

That the selected dose of indomethacin is adequate to suppress intrarenal i-PG production is evident in Figure 1. Reductions in i-PG effluent were observed in all of the remaining groups receiving indomethacin. Clearly, indomethacin administration resulted in parallel reductions in i-PG efflux and circulating renin in both filtering and nonfiltering kidneys (Figs. 1 and 5). Indomethacin also suppresses renin in anesthetized rats (Leyssac et al., 1975), in conscious rabbits (Romero et al., 1976), in anesthetized dogs (Yun et al., 1977), and in man (Fröhlich et al., 1976a). Although the present study...
provides evidence that indomethacin suppresses PRA in renally denervated one-kidney dogs as well, and, by inference, through an intrarenal action, precise mechanisms are elusive.

In series 2, renal perfusion pressure was decreased to approximately 60 mm Hg in both control and indomethacin-treated dogs. Previous studies have shown that comparable suprarenal aortic constrictions in intact anesthetized dogs produced a significant rise in renin activity (Schmid, 1972). In the present study, both the control and the i-PG-inhibited denervated kidneys responded to the reduced renal perfusion with similar PRA increases, despite the fact that initial renal venous i-PG effluent from the indomethacin-treated kidneys was significantly less than the efflux from the control series. Therefore, if intrarenal PG synthesis does modify renin mechanisms, that relationship is disassociated during reduced renal perfusion pressure.

Reduction of renal perfusion pressure produced no significant increases in the i-PG effluent measured in either control or indomethacin-treated kidneys. McGiff et al. (1970) reported an increase in renal venous PG concentration during similar renal ischemia. However, PG output was assayed by the blood-superfused organ technique, a method which does not allow for the dilutional changes resulting from the compromised RBF during pressure reduction. A more recent study (Beckman and Zehr, 1975) concluded that, although renal venous and urinary PGE concentrations increased during ischemia, secretion and urinary excretion rates were not increased. The present data support these conclusions. A significant decrease ($P < 0.05$) was noted in i-PG effluent from the indomethacin-treated kidney during the aortic constriction. Since the rate of indomethacin delivery was constant, this reduction in i-PG output could have resulted from an increase in the concentration of the inhibitor in the renal mass as the RBF was lowered. If medullary PG synthesis remains constant during renal ischemia, then intrarenal concentrations of PG's or their synthetic intermediates will be increased due to a reduced rate of washout. Indeed, in the present study, actual renal venous concentrations of i-PG invariably were increased provided that indomethacin was not administered. One might then argue that an increased medullary concentration of PG's or their synthetic intermediates might in some undefined way be partially responsible for the increased renin during renal ischemia. There have been several reports that arachidonic acid and components of prostaglandin synthetic pathways enhance renin release both in whole kidneys (Bolger et al., 1976; Larsson et al., 1974) and in in vitro slices (Whorton et al., 1977; Weber et al., 1976). It seems clear from the present study that increased intrarenal i-PG concentrations during renal ischemia are not responsible for the associated increase in renin because the response to ischemia remained despite the documented suppression of i-PG efflux with indomethacin.

Even though the results of series 2 suggest that baroreceptor-mediated renin release is independent of intrarenal PG production, the conclusion must be qualified because the aortic clamp altered GFR in the control group and, although data were not available, probably reduced GFR in the indomethacin group as well. As a result, sodium delivery to the macula densa was most likely diminished during the ischemia. According to a generally accepted theory, the decrease in Na$^+$ transport at the macula densa would elicit an increase in renin secretion. The renin response to aortic constriction may have resulted in part, from stimulation of tubular receptors as well as the intrarenal baroreceptor. In series 3, furosemide (a 5 mg/kg bolus plus a 1 mg/kg per hr infusion) was administered to control and to PG-inhibited dogs. Previous studies have demonstrated that this diuretic stimulates renin release in intact anesthetized dogs. Vander and Carlson (1969) postulated that furosemide inhibits sodium transport to the macula densa cells. The reduction of Na$^+$ transport was hypothesized to enhance renin release during furosemide administration. However, Corsini et al. (1975) have suggested that furosemide may act on both tubular and baroreceptor mechanisms. Intact anesthetized dogs typically respond to furosemide with an increase in renin release, Na$^+$ excretion, and RBF, and a decrease in GFR (Vander and Carlson, 1969; Bailie et al., 1973). Similar renal responses, with the exception of the increased RBF, were observed in the present study.

The renin response to the diuretic in the control dogs was significantly greater than in those with the indomethacin-blocked kidney and is similar to the blunting of the furosemide-induced renin release reported in intact rabbits (Romero et al., 1976) and in man (Frolich et al., 1976). These data imply that intrarenal PG synthesis modifies renin secretion stimulated via tubular mechanisms. However, considering the evidence that furosemide redistributes intrarenal RBF (Birch et al., 1967), and even the untested possibility of a direct effect of furosemide on the juxtaglomerular cells, the present experiment does not rule out a possible indomethacin or PG mediation of renin release via other more direct mechanisms. In fact, a recent report (Weber et al., 1977) has shown that, in man, administration of furosemide is accompanied by concomitant increases of free arachidonic acid and renin, and it was proposed that the release of this primary PG precursor was in some way responsible for the renin response to the diuretic.

The possibility that indomethacin may influence the release of renin stimulated by $\beta$-adrenergic agonists was also investigated. The present finding that isoproterenol did not change renal venous i-PG efflux is consistent with the data from isolated perfused rabbit kidneys (Needleman et al., 1974). In both control and indomethacin treated kidneys,
PRA increased following isoproterenol infusion (Fig. 4). Intravenous isoproterenol has previously been shown to induce renin release in one-kidney dogs (Johnson et al., 1976). In the present study, the isoproterenol-induced renin response in indomethacin-treated dogs was less numerically than in the saline-infused controls; however, there was no statistically significant difference between the response of the two groups in terms of either absolute or percent change (P > 0.2).

Interpretation of these data is complicated by the decrease in mean arterial pressure induced by isoproterenol in both the control and indomethacin-treated dogs (Table 4). Significant falls in RBF and GFR accompanied the pressure drops in both groups. These changes may have enhanced renin secretion via intrarenal baroreceptors and tubular mechanisms independent of β-mediation. In addition, serum K⁺ concentration and K⁺ excretion rates decreased after isoproterenol infusion into dogs of both groups. Hypokalemia, which may develop during isoproterenol treatment, stimulates renin secretion through tubular mechanisms rather than through adrenergic receptors. Changes in mean pressure, RBF, GFR, and intrarenal K⁺ levels may have masked the true effects of indomethacin on β-mediated renin release in the present experiment. Firm conclusions concerning the effect of indomethacin on β-stimulated renin secretion must await the results of investigations in which the hormone is not influenced by intrarenal baroreceptors, the macula densa, and changes in humoral factors.

The suppression of basal renin secretion and of the furosemide-induced release could possibly be explained by a direct toxic effect of indomethacin on the juxtaglomerular cells rather than by a decrease in PG production. There are several lines of indirect evidence against indomethacin toxicity. Reductions of renin have been achieved with structurally different PG inhibitors (Romero et al., 1976). In addition, in the present study, comparable increases in PRA were observed in both control and indomethacin-treated dogs in response to reduced renal perfusion pressure, an unlikely response had indomethacin had a direct toxic effect.

How then does indomethacin administration affect in vivo reductions in renin output? The answer to this question appears to lie in the dogs of series 5. Both the filtering and nonfiltering kidneys responded to indomethacin (Figs. 1 and 5), which argues that the response is not dependent on macula densa mechanisms. However, baroreceptor mechanisms could not be ruled out completely, especially in view of a recent report (Data et al., 1978) that appears to implicate a cyclooxygenase-dependent component of the renal baroreceptor. Prior blockade of the renal baroreceptor by intrarenal papaverine infusion in dogs of protocol C (series 5) failed to prevent the rise in renin secretion (Fig. 7) during intrarenal arachidonic acid infusion. Thus, by elimination, direct in vivo modification of juxtaglomerular cell secretory activity by PG-related synthetic events seems a reasonable conclusion. The failure of indomethacin to block renin secretion in the papaverine-treated dogs even though i-PG was reduced (Fig. 7) may be related to the fact that papaverine is a potent inhibitor of cyclic nucleotide phosphodiesterase activity (Nickerson, 1975). The high level of renal venous cAMP in this group (Table 7) is probably a reflection of this action. Although the precise role that cAMP may play in renin secretory mechanisms is still under question, it has been reported that renin is stimulated by this nucleotide both in vivo (Winer et al., 1971) and in vitro (Michelakis et al., 1969). If cAMP had played a role in renin secretion here, the documented elevation of the nucleotide (Table 7) may have overridden any suppressive influence of reduced PG synthetic activity during indomethacin infusion in the papaverine-treated kidneys. The data of series 5 imply that a direct action on juxtaglomerular cells by products of arachidonic acid-cyclooxygenase is the primary in vivo mechanism for PG-renin interaction, a response analogous to that reported in vitro (Weber et al., 1975; Whorton et al., 1977). The present data also seem to argue against prostaglandin-related modification of renal baroreceptor or β-adrenergic receptor mechanisms because renin was shown to increase in the face of suppressed i-PG and, presumably, intrarenal synthetic events. Since indomethacin is effective in blunting the renin response to furosemide, one could argue that PG synthesis modifies tubular control of renin. However, i-PG secretion (if it is indeed an index of intrarenal PG synthetic events) was unchanged by furosemide in both the control and indomethacin-treated kidney. Therefore, the renin-PG relationship again is dissociated during renin stimulation by this diuretic, and one could scarcely argue that the renin response to furosemide is mediated by PG-dependent events. In light of the above, it seems reasonable to conclude that the in vivo PG-synthetic modification of renin release is probably via direct modification of the juxtaglomerular cells rather than via modification of the major renin control mechanisms previously described (Davis and Freeman, 1976). Since the major site of PG synthesis is generally accepted to be medullary, whereas renin secretion is predominantly cortical, the question of the mechanical coupling between arachidonic acid-cyclooxygenase products and renin secretion remains. In the filtering kidney, the lipid nature and high partition coefficient of PG's should allow rapid movement into the tubular and vascular compartments with subsequent transport from medullary to cortical regions. However, as suggested by Data et al. (1978), it is doubtful that movement via the tubular system was a major factor in the nonfiltering kidneys, especially since the measured glomerular filtration in our dogs was only 1 ml/min. This would leave medullary to cortical transport primarily dependent on the vasa recta vascular network. Alternatively,
significant cyclooxygenase activity has been reported to reside in cortical tissue (Larsson and Anggård, 1973) and thus medullary to cortical movement of PG synthetic products need not be invoked.

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References


Li JCR (1964) Statistical Inference. Ann Arbor, Michigan, Edwards Brothers, pp 505-526


Influence of renal prostaglandin synthesis on renin control mechanisms in the dog.

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