Effect of Furosemide on Renal Function in the Stenotic and Contralateral Kidneys of Patients with Renovascular Hypertension

Luis Ruizlope, M.D., Rafael Garcia-Robles, M.D., Jose Sancho-Rof, M.D., Carlos Paya, M.D., Jose L. Rodicio, M.D., Cameron G. Strong, M.D., Franklyn G. Knox, M.D., Ph.D., and J. Carlos Romero, M.D.

SUMMARY In a group of six patients diagnosed as having unilateral renovascular hypertension due to fibromuscular dysplasia, inulin glomerular filtration rate, (GFR) and PAH renal plasma flow, (RPF) clearances, urine flow (V), urine sodium (UVNa), potassium (UVK), urinary excretion of prostaglandin E\(_2\) (UV\(_{\text{PGE}}\)), thromboxane B\(_2\) (UV\(_{\text{TB}}\)), and 6-keto prostaglandin F\(_{1\alpha}\) (UV\(_{\text{6-keto-PGF}_{1\alpha}}\)) were measured in each kidney before and after the i.v. administration of furosemide (20 mg). The basal values of GFR, RPF, UV\(_{\text{Na}}\), UV\(_{\text{PGE}}\), UV\(_{\text{TB}}\), and UV\(_{\text{6-keto-PGF}_{1\alpha}}\) were lower (p < 0.01) in the stenotic kidney. Furosemide increased RPF 11% and 50%, GFR 25% and 62%, and V 142% and 280% in the contralateral and stenotic kidney respectively. The increase of UV\(_{\text{Na}}\) was similar in the two kidneys. In the stenotic kidney, both UV\(_{\text{PGE}}\) and UV\(_{\text{6-keto-PGF}_{1\alpha}}\) increased significantly (p < 0.01) with furosemide while UV\(_{\text{TB}}\) remained unchanged. Furosemide did not alter the rate of excretion of the three prostaglandins measured in the contralateral kidney. We conclude that furosemide significantly improves renal circulatory and excretory function of the stenotic kidney. Since prostaglandin excretions also increased, the vasodilatation in the stenotic kidney may be prostaglandin mediated.

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KEY WORDS • hypertension, renovascular • PGE\(_2\) • TxB\(_2\) • 6-keto-PGF\(_1\)
• furosemide • Howard test

It is known that the vasodilator action of furosemide in the normal kidney is mostly mediated by increased synthesis of prostaglandins,\(^1\)-\(^4\) which may also modulate the diuretic effects of furosemide.\(^\text{4}\) Furosemide is currently used in patients with unilateral renovascular stenosis; however, it is not known whether its hemodynamic and diuretic effects are equally exerted or predominantly exerted in the stenotic kidney or in the contralateral kidney. Further, we have no knowledge about the changes induced by furosemide in prostaglandin synthesis in the stenotic and contralateral kidney.\(^\text{5,6}\) In a recent survey of the literature,\(^\text{7}\) there is convincing evidence supporting the notion that increased synthesis of prostaglandins is important in the maintenance of renal blood flow during the early phases of renovascular hypertension. However, in the chronic stages of hypertension, the role of prostaglandins has not been adequately explored.\(^\text{7}\)

This study was undertaken to determine the effect of furosemide on renal hemodynamics, renal excretory function, and prostaglandin excretion in both the stenotic and contralateral kidneys of patients with renovascular hypertension.

Materials and Methods

Patient Protocol

The study was conducted in six patients (two men and four women between 20 and 34 years of age), diagnosed as having arterial hypertension (blood pressure above 150/95 on three separate readings with a mercury sphygmomanometer) in whom the renal arteriogram showed the typical findings of unilateral renal artery stenosis due to fibromuscular dysplasia. All patients had a renal vein renin ratio above 2 and contralateral suppression of renin secretion according to the criteria of Vaughan, et al.\(^\text{8}\) The patients' consent was obtained and they were admitted to the hospital. Hypo-
tensive medication was discontinued for 2 weeks prior to the study. Medication was reinstalled whenever the levels of diastolic blood pressure exceeded 110 mm Hg, in which case the patient was removed from the study. On the day of the study, a cystoscopy and bilateral retrograde ureteral catheterization were performed. A priming injection of insulin and paraaminohippurate was followed by continuous infusion of both substances at standard doses through an antecubital vein cannula. All these maneuvers were performed without altering the early morning recumbent position. After a period of stabilization, urine was collected from both kidneys during 30 minutes and a blood sample was drawn in the middle of the period in order to obtain an initial measurement of the glomerular filtration rate (GFR), renal plasma flow (RPF), urine volume (V), natriuresis (U_Na), kaliuresis (U_K), and the urinary excretion of prostaglandin E₂ (PGE₂), thromboxane B₂ (TXB₂), and 6-keto-PGF₁₀α. At the end of this initial period, furosemide (20 mg i.v.) was administered and 15 minutes later the previous procedure was repeated.

### Table 1. Effect of Furosemide on Renal Hemodynamics and Excretory Function

<table>
<thead>
<tr>
<th></th>
<th>Urine flow (ml/min)</th>
<th></th>
<th>Urine sodium (µEq/min)</th>
<th></th>
<th>Urine potassium (µEq/min)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Contra-lateral</td>
<td>Stenosed</td>
<td>Contra-lateral</td>
<td>Stenosed</td>
<td>Contra-lateral</td>
<td>Stenosed</td>
</tr>
<tr>
<td>Initial</td>
<td>6.1 ± 4.2</td>
<td>1.0 ± 1.1*</td>
<td>319.7 ± 329.6</td>
<td>110.7 ± 196.3*</td>
<td>82.1 ± 35.3</td>
<td>38.8 ± 41.3</td>
</tr>
<tr>
<td>After furosemide</td>
<td>14.8 ± 6.6</td>
<td>3.8 ± 3.2t</td>
<td>1595.7 ± 632.0</td>
<td>474.7 ± 436t</td>
<td>204.7 ± 56.8</td>
<td>60.2 ± 38.2*</td>
</tr>
<tr>
<td>ρ</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
<td>NS</td>
</tr>
</tbody>
</table>

*p<0.01 vs contralateral.

Values expressed as means ± SD.

**†p <0.025 vs contralateral.**

Finally, prostaglandins were eluted with 10 ml of petroleum ether passed to remove nonpolar lipids and monohydroxy fatty acids. Finally, prostaglandins were eluted with 10 ml of methyl formate or acetonitrile. We found that both solvents yielded similar recoveries. However, elution with acetonitrile facilitated immediate determinations in HPLC since this solvent is currently used as the mobile phase in isocratic determinations. The fraction containing prostaglandins were then dried under N₂ and reconstituted in phosphate buffer for radioimmunoassay with a technique similar to that published elsewhere.¹⁰,¹¹

Total recoveries for the three prostaglandin assays ranged from 81% to 87% with a variation coefficient of 8.9%.

Minimum detection levels of radioimmunoassay varied from 3 to 4 pg/ml for PGE₂, 2.5 to 5 pg/ml for 6-keto-PGF₁₀α, 5 to 10 pg/ml for TXB₂. The sensitivity of radioimmunoassay, which was estimated as the minimal variation in the concentration of prostaglandins to produce 10% displacement of each labeled PG from each specific antibody within the steepest portion of the curve, varied from 8.1 to 12.3 pg/ml.

Antibodies for PGE₂ were purchased at the Pasteur Institute. Antibodies for TXB₂ and 6-keto-PGF₁₀α were obtained in our laboratories. Cross reactivity of these antibodies with other prostanoids, arachidonic acid, arachidonic acid, endoperoxide analog H₂, PGE₂, PGD₂, PGF₂α, PGE₁, 13-14 dihydro-15-keto-E₂, F₂α, varied from 0 to 3%. The specificity of radioimmunoassays was further checked by pooling samples of urine from three patients exhibiting the higher concentration of prostaglandins being measured. Radioimmunoassay was performed in these samples after octadeylsyl chromatography, that is, before HPLC and after HPLC. Differences between radioimmunoassay and HPLC (Beckman M110A, Beckman Instruments, Berkeley, California) determinations range from 4% to 9%. Minimum detection levels of HPLC determinations with Altex C18 column (Beckman) and 165 Beckman UV detector were 25 ng/ml for TXB₂, 45 ng/ml for PGE₂, and 10 ng/ml for 6-keto-PGF₁₀α. That the HPLC peaks obtained in this urine pool corresponded to PGE₂ and 6-keto-PGF₁₀α was further ascertained by determinations of these compounds with helium gas chromatography (Carlo Erba, Milan, Italy), mass spectrometry (Kratos M550, Manchester, England) using a method similar to that published by Barrow, et al.¹³

Statistical studies were done using the paired Student’s t test and the Wilcoxon test for unpaired data. Determinations of plasma renin activity (PRA) in renal veins from both kidneys and in the abdominal aorta below the renal arteries were performed 10 to 20 days prior to the study following the method of Haber.¹⁴

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### Chemical Determinations

Insulin and paraaminohippurate were measured by standard techniques. Sodium and potassium were measured with an IL143 Digital Flame Photometer (Instrumentation Laboratories, Lexington, Massachusetts).

Urine concentrations of PGE₂, 6-keto-PGF₁₀α, and TXB₂ were determined by radioimmunoassay after the separation method of Powell. This consisted of centrifugation of urine samples for 5 minutes at 750 g after which the pH was adjusted to 3 with 1 N solution of citric acid. Octadeylsyl columns (Sep-Pack C-18, Waters Associates, Milford, Massachusetts) were prepared by washing them first with HPLC-graded distilled water and then with 95% distilled ethanol. The acidified urine samples diluted with equal volume of triple-distilled H₂O were then passed through this column. Phospholipids, proteins, and very polar materials either were not retained by the octadeylsyl column or were eluted with 10 ml of diluted 5% ethanol. After ethanol, 10 ml of petroleum ether was passed to remove nonpolar lipids and monohydroxy fatty acids. Finally, prostaglandins were eluted with 10 ml of methyl formate or acetonitrile. We found that both solvents yielded similar recoveries. However, elution with acetonitrile facilitated immediate determinations in HPLC since this solvent is currently used as the mobile phase in isocratic determinations. The fraction containing prostaglandins were then dried under N₂ and reconstituted in phosphate buffer for radioimmunoassay with a technique similar to that published elsewhere.¹⁰,¹¹

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modified as published elsewhere. These determinations are routinely done in renovascular hypertensive patients to conform to the score of surgical curability proposed by Vaughan et al.

Results

As shown in table 1, during the control period the CPAH and CIN in the stenotic kidney were 74.6% and 73.6% lower than in the contralateral kidney. Filtration fractions were 23.1% for the contralateral and 24.0% for the stenotic kidney. Furosemide significantly increased CPAH in both kidneys; however, the percentage increase was more pronounced in the stenotic kidney (50.6%) than in the contralateral kidney (11.6%). Since these changes were accompanied by a proportional increment of CIN in the stenotic (62.5%) and in the contralateral kidney (25.7%), the filtration fractions remained constant, 25.9% and 26.0%. Plasma Na concentrations were within the normal range (140 ± 2 mEq/liter) before and during furosemide. Sodium excretion was 65.3% lower in the stenotic kidney than in the contralateral kidney. Sodium concentration in the urine from the stenotic kidney was twice that in the urine from the contralateral kidney. Furosemide induced a 4.2- and 5-fold increase of sodium excretion in the stenotic and contralateral kidney, respectively. These increments were accompanied by a proportional increase of urine volume in the stenotic kidney, but in the contralateral kidney the increase in urine volume was 50% less than the percentage increase of sodium resulting in increased urine sodium concentration. As shown in table 1, there was a significantly higher rate of potassium excretion in the contralateral kidney than in the stenotic kidney. Furosemide significantly increased the excretion rate of this cation in both kidneys.

As shown in table 2, the excretion rates of PGE2, TxB2, and 6-keto-PGF1α were significantly higher in the contralateral than the stenotic kidney. There was also a great variability in the three prostanoids' rate of secretion from patient to patient. Furosemide significantly increased the secretory rate of PGE2 and 6-keto-PGF1α only in the stenotic kidney. In the contralateral kidney, prostanoids' excretory rates were not further affected by furosemide. Furosemide failed to alter the excretory rates of TxB2 in both kidneys. Table 3 shows that the average values of peripheral PRA measured in these patients, either after 24 hours of recumbency or after 2 hours of ambulation, were above the normal range, which was 0.8 to 2.1 ng/ml/hr and 2.3 to 5.2 ng/ml/hr respectively. In addition, all the patients exhibited a significant lateralization of renin on the stenotic side, with a stenotic/nonstenotic ratio higher than 2.8 having a strong surgical indication according to the score of Vaughan et al.

Discussion

Split renal functional studies were first introduced 3 decades ago and advocated as a valuable tool in the recognition of an ischemic kidney and in the prediction of the surgical outcome. The initial studies described significant reductions in RBF, GFR, urine volume, and sodium excretion in the stenotic kidney of a magnitude similar to those reported in this study. Under these conditions we found that a single intravenous injection of furosemide induced percentage increases in RBF and GFR which were 40% and 36.8% respectively higher in the stenotic than the contralateral kidney. This lateralization of the effects of furosemide should be regarded as highly significant if one considers that, because of the existing differences of RBF, the stenotic kidney received much less furosemide than the contralateral kidney.

An interesting finding of this study was the higher excretory rates of the three prostanoids from the contralateral side. This agrees with a previous report of Juncos and Strong who found a higher concentration

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Effect of Furosemide on Urinary Excretion of Prostaglandins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PGE2 (pg/min)</td>
</tr>
<tr>
<td></td>
<td>Contra-</td>
</tr>
<tr>
<td>Initial</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CIN</td>
</tr>
<tr>
<td>Initial</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2117 ± 2344</td>
</tr>
<tr>
<td>After furosemide</td>
<td>1796 ± 1726</td>
</tr>
<tr>
<td>ρ</td>
<td>NS</td>
</tr>
</tbody>
</table>

*p<0.01 vs contralateral kidney.
Values expressed as means ± sd.

PGE2 = prostaglandin E2; TxB2 = thromboxane B2; 6 keto F1α = prostaglandin 6 keto F1α.
of radioimmunoassayable PGE₂ in the renal venous effluent of the contralateral kidney in hypertensive patients with unilateral renal stenosis. Data on changes in renin synthesis of prostaglandins after unilateral renal stenosis have focused on defining the acute events triggered in both kidneys by unilateral renal arterial constriction, but attempts to evaluate the release of prostaglandins in chronic experiments or human hypertension have been difficult to carry out.

It is known that acute constriction of the renal artery induces an increase in renal prostaglandins, which in the early stages of hypertension appears to play an important role in protecting the renal circulation and thereby renal function. However, McGiff, et al., have reported that unilateral renal arterial constriction is also followed 2 minutes later by an increase in renin release and circulating angiotensin, which triggers the synthesis and release of prostaglandins in the contralateral kidney about 7 minutes after arterial constriction. This notion was supported by the finding that the threshold concentration of angiotensin II needed to release prostaglandins from the kidney was in the range of that reported in patients with renovascular hypertension. Further, Galvez et al. blocked the contralateral diuresis that follows ipsilateral arterial constriction with either angiotensin antagonists or blockers of prostaglandin synthesis. Thus, as hypertension progresses, ischemia in the stenotic kidney is diminished by a higher perfusion pressure, rendering the stenotic kidney less dependent on synthesis of prostaglandins. Since renin release continues, prostaglandin synthesis may be maximally stimulated in the contralateral kidney because of the high levels of angiotensin.

Our results could be interpreted as indicating that furosemide failed to induce a marked increase in prostaglandin E₂ and 6-keto-PGF₁α production in the contralateral kidney because of the already high levels of production. However, furosemide produced a 2.5- to 5-fold increase of these prostanoids in the stenotic kidney. It is known that in the normal kidney the furosemide-induced vasodilatation is prostaglandin-dependent since it could be prevented by blocking prostaglandin synthesis with antiinflammatory drugs. Although the increase in prostaglandin excretion could represent a washout in response to increased urine flow in the stenotic kidney, this is not likely since increased urine flow did not increase prostaglandin excretion in the contralateral kidney. Thus, the increased excretion probably represented increased synthesis of prostaglandins, provided that furosemide did not produce unequal interferences in each kidney in the organic acid transport system responsible for the excretion of both PAH and prostaglandins. Within these limitations, the increase in prostaglandin synthesis in the stenotic kidney may be viewed as responsible for the greater vasodilatation than in the contralateral kidney in which urinary prostaglandins did not increase. It has been reported that furosemide produces an increase of urine excretion of kallikrein. However, it remains uncertain as to what extent this effect is related to the observed increases in renal blood flow, because urine kallikrein is also increased by diuretics such as thiazides and acetazolamides that do not produce renal vasodilation.

In contrast, most of the natriuretic action of furosemide is not the result of renal vasodilation but the consequence of an effective blockade of sodium chloride reabsorption in the loop of Henle. This action was similar in both kidneys since urine excretion was increased by 4.2-fold in the stenotic kidney and by 5-fold in the contralateral kidney. The 26% higher natriuresis of the contralateral kidney can account for the differences in furosemide uptake due to 25.4% differences in RBF during control conditions. We have no explanation for the increased levels of TxB₂, in the contralateral kidney during control conditions. Renal tissue produces small amounts of TxA₂ as compared to other prostanoids. However, the phenomenon may reflect increased platelet adherence to arterial endothelial lesions induced by constant exposure to both high arterial pressure and angioten-

### Table 3. Values of Plasma Renin Activity in Six Cases of Unilateral Renovascular Hypertension

<table>
<thead>
<tr>
<th>Case No.</th>
<th>PRA 1</th>
<th>PRA 2</th>
<th>Renal vein contralat</th>
<th>Renal vein stenotic</th>
<th>Aorta</th>
<th>Contralat</th>
<th>A</th>
<th>Stenotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.5</td>
<td>12.6</td>
<td>8.8</td>
<td>21.6</td>
<td>7.9</td>
<td>0.11</td>
<td>1.73</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.0</td>
<td>7.9</td>
<td>4.6</td>
<td>14.6</td>
<td>5.2</td>
<td>0.11</td>
<td>1.80</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3.6</td>
<td>14.7</td>
<td>6.3</td>
<td>18.7</td>
<td>6.5</td>
<td>0.03</td>
<td>1.87</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>3.9</td>
<td>10.8</td>
<td>4.1</td>
<td>14.0</td>
<td>3.9</td>
<td>0.05</td>
<td>2.58</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3.2</td>
<td>8.9</td>
<td>6.2</td>
<td>15.2</td>
<td>6.3</td>
<td>0.01</td>
<td>2.58</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2.3</td>
<td>8.5</td>
<td>3.2</td>
<td>8.9</td>
<td>4.0</td>
<td>0.20</td>
<td>1.12</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>3.25</td>
<td>10.57</td>
<td>5.53</td>
<td>15.50</td>
<td>5.63</td>
<td>0.085</td>
<td>1.95</td>
<td></td>
</tr>
<tr>
<td>SE</td>
<td>0.39</td>
<td>1.09</td>
<td>0.81</td>
<td>1.77</td>
<td>0.64</td>
<td>0.029</td>
<td>0.23</td>
<td></td>
</tr>
</tbody>
</table>

PRA expressed as ng/ml/hr.
PRA 1 = level after overnight recumbency; normal values = 0.8-2.1.
PRA 2 = level after 2 hours of ambulation; normal values = 2.3-5.2.
V = PRA renal vein.
A = PRA aorta.
sin. It has been recently reported that furosemide induces increased synthesis of PGI₂ in endothelial cells. However, in unpublished observations we have failed to alter platelet production of prostaglandins. This could explain the higher levels of TxB₂ excretion in the contralateral kidney, which remained unaltered after administration of furosemide.

In summary, the findings of this study have therapeutic implications since they advocate the use of furosemide to improve renal circulation and renal function of the stenotic kidney in patients with renovascular hypertension. The preferential vasodilative effect of furosemide in the stenotic kidney could be mediated by an increase in PGF₂α and PGI₁, because the urine excretory rates of PGF₂α and 6-keto-PGF₁α were significantly reduced in the stenotic kidney and remained unchanged in the contralateral kidney.

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References
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