Furosemide Increases Urine 6-Keto-Prostaglandin F\textsubscript{1α}

Relation to Natriuresis, Vasodilation, and Renin Release

THOMAS W. WILSON, M.D., C. BOYD LOADHOLT, PH.D.,
PHILIP J. PRIVITERA, PH.D., AND PERRY V. HALUSHKA, M.D., PH.D.

SUMMARY While previous studies have shown that prostaglandins (PG) mediate the renal vasodilating and renin-releasing actions of furosemide, the dose-response relationship has not been defined nor has the specific PG involved. Prostacyclin (PGI\textsubscript{2}) is synthesized in the renal cortex, is a vasodilator, can release renin and, therefore, could mediate these actions of furosemide. The authors measured urinary excretion of the PGI\textsubscript{2} hydrolysis product 6-keto prostaglandin F\textsubscript{1α} (U6kV) in response to increasing intravenous bolus doses of furosemide in anesthetized dogs. Furosemide 0.1 mg/kg increased urine volume (V) and sodium excretion (UNaV) compared to control dogs, but did not change U6kV, p-aminohippurate clearance (CPAH), or plasma renin activity (PRA). A higher dose (1.0 mg/kg) increased V, UNaV, U6kV (23.4 ± 11.0 to 56.5 ± 18.7 ng/15 min, \( p < 0.05 \)), CPAH (121 ± 40 to 304 ± 80 ml/min, \( p < 0.05 \)), and PRA (5.4 ± 2.2 to 11.7 ± 4.4, \( p < 0.05 \)). There was no correlation between U6kV and V or UNaV, but correlations existed between U6kV and CPAH (\( r = 0.81, p < 0.001 \)) and between the percent increase of U6kV and the percent increase of PRA following each dose of furosemide (\( r = 0.59, p < 0.05 \)). In separate experiments, we assessed the effects of increasing renal blood flow (RBF) on U6kV, by infusing the PG-independent vasodilator secretin into a renal artery. RBF of the single kidney increased from 124 ± 32 to 202 ± 21 ml/min (\( p < 0.01 \)) and CPAH from 56 ± 18 to 83 ± 42 ml/min (\( p < 0.05 \)), but U6kV did not change. Indomethacin reduced U6kV but did not affect secretin-induced increases in RBF or CPAH. The results indicate that furosemide-induced natriuresis occurs at low doses and in the absence of an effect on U6kV. In contrast, furosemide-induced vasodilation and renin release required higher doses and were associated with increased U6kV. Because secretin can increase RBF without changing U6kV, we suggest that furosemide-induced increases in U6kV are not secondary to increased RBF. Rather, it appears that furosemide releases renal PGI\textsubscript{2} which, in part, mediates vasodilation and renin release, and was excreted in urine as 6-keto-prostaglandin F\textsubscript{1α} (Hypertension 4: 634-641, 1982)

KEY WORDS • furosemide • renal blood flow • plasma renin activity • renal prostaglandins • prostacyclin • indomethacin • secretin

Furosemide causes natriuresis, renal vasodilation, and renin release\textsuperscript{1-3} in addition to increasing renal prostaglandin (PG) production.\textsuperscript{4-6} The interrelationship of these actions has been the subject of numerous investigations. While furosemide-induced renal PG synthesis was initially thought to mediate all the other effects, it appears that natriuresis may be independent of PGs. Oral furosemide produces a marked increase of sodium excretion without increasing PGE excretion,\textsuperscript{7} while indomethacin blocks furosemide-induced PG synthesis but does not affect the natriuretic response.\textsuperscript{8-9} On the other hand, PG synthesis inhibition does blunt furosemide-induced vasodilation and renin release.\textsuperscript{10-12} Whether dose requirements for these various actions of furosemide are different is unknown as previous studies have all used a single large bolus (5 mg/kg or more) or infusion rate.

Furosemide appears to increase renal PG synthesis by increasing the release of the precursor, arachidonic acid,\textsuperscript{13-14} thereby increasing the synthesis of many PGs.\textsuperscript{15} As it is known that the kidney can synthesize PGE\textsubscript{2}, PGF\textsubscript{2α}, PGD\textsubscript{2}, thromboxane A\textsubscript{2}, and prostacyclin (PGI\textsubscript{2}),\textsuperscript{16-18} the question arises as to which of these mediates vasodilation and renin release. Most studies have focused on PGE\textsubscript{2}.\textsuperscript{3-5,7} but there are indications that

\[634\]
it may not be the PG of physiologic significance for these actions. For example, PGE₂ is much less potent than PG₁ in stimulating renin release. Second, while intrarenal infusion of PGE₂ does increase renal blood flow, administration of PGE₂ in large doses does not prevent or completely reverse the decrease in renal blood flow in anesthetized animals caused by PG₁ synthesis inhibitors. Third, urine PGE₂ excretion, often taken as a measure of renal PGE₂ synthesis, may be affected by urine volume, and therefore such excretion may be difficult to interpret when urine volume is increased by furosemide.

On the other hand, PG₁ is a potent vasodilator, and is a major renal cortical PG. Because vasodilatation and renin release are "cortical events," we proposed that PG₁ might mediate these actions. Proof of this supposition is difficult because of the lack of specific inhibitors of PG₁ synthesis or action. In addition, the short half-life of PG₁ at physiologic pH precludes direct measurement. It is hydrolyzed nonenzymatically to, and is the only known precursor of, 6-keto-prostaglandin F₁α (6-ketoPGF₁α). This material has been detected in urine and plasma but the relative contribution to the urine from the kidney is unknown.

In this study, we determined the dose-response relationships for furosemide-induced diuresis, natriuresis, vasodilatation, renin release, and increases in urinary 6-ketoPGF₁α in dogs. In another group of dogs we increased renal blood flow with secretin, a PG independent agent, to assess the effects of renal vasodilatation per se on urinary excretion of 6-ketoPGF₁α.

### Methods

**Furosemide Studies**

Nine mongrel dogs of both sexes weighing 7.3 to 21.0 kg were used. Drinking water was replaced with 0.9% sodium chloride for 18 to 24 hours prior to the experiments. The dogs consumed between 1 and 2 liters. Anesthesia was induced with pentobarbital 30 mg/kg intravenously and maintained as necessary with additional doses of 4 to 6 mg/kg. The dogs were intubated and ventilated with a Harvard Respirator (Harvard Apparatus, Dover, Massachusetts) and maintained at constant temperature with a heating pad.

Heart rate (HR) was obtained from the electrocardiogram. Femoral artery blood pressure (MAP) was recorded via a Statham model P23Dc pressure transducer (Statham, Hato Rey, Puerto Rico) on a Grass Model 7 polygraph (Quincy, Massachusetts). A catheter was placed in the right atrium via the jugular vein for blood sampling. The urethra of males was catheterized with a pediatric nasogastric feeding tube and that of females with a No. 8 Foley catheter. Both femoral veins were cannulated for the infusion of p-aminohippurate (PAH; Merck, Sharp and Dohme, Rahway, New Jersey), creatinine (Fisher Scientific, Fair Lawn, New Jersey), and fluid replacement solution (see below).

PAH was infused at 3 mg/min and creatinine at 9 mg/min (Harvard Infusion Pump, Harvard Apparatus) to maintain serum concentrations of 1 to 3 mg/dl and 5 to 15 mg/dl respectively. To offset as much as possible the stimulating effects of anesthesia on PRA and PG synthesis, the dogs were volume-loaded with about 75 ml/kg intravenously of a solution containing 80 mEq/liter sodium, 20 mEq/liter potassium, and 100 mEq/liter chloride over 1 hour. Urinary losses throughout the experiment were replaced with this solution.

Furosemide (a generous gift of Hoechst Roussel Ltd., Somerville, New Jersey) and indomethacin (Sigma Chemicals Ltd., St. Louis, Missouri) were dissolved in 0.1 M sodium diphosphate buffer, pH 8.5, immediately before use.

The actual experiments were begun about 1 hour after the completion of the volume-loading procedure and consisted of 17 consecutive 15-minute urine collection periods. Periods 1 through 4 served as controls.

Six dogs (the furosemide group) received furosemide in 2 ml buffer as an intravenous bolus: 0.01 mg/kg at the end of Period 4, 0.1 mg/kg at the end of Period 8, and 1.0 mg/kg at the end of Period 12. Three dogs (the control group) received buffer (vehicle) only at these times.

Blood samples were taken at the midpoints of Periods 1, 2, 4, 5, 8, 9, 12, 13, and 17. Indomethacin 10 mg/kg dissolved in buffer (2–3 mg/ml) was infused intravenously over 5 minutes at the end of Period 16.

Blood was allowed to clot at room temperature, and serum was immediately separated for the determination of serum sodium, potassium, creatinine, and PAH. Blood for plasma renin activity (PRA) was collected in Vacutainer tubes containing disodium EDTA and immediately cooled on ice before the plasma was separated in a refrigerated centrifuge and stored at −20°C until assay. A microhematocrit was determined for each blood sample.

Urine was collected in polypropylene bottles, an aliquot removed for determination of sodium, potassium, creatinine, and PAH, and the remainder stored at −20°C for up to 2 weeks until assayed for 6-ketoPGF₁α. Storage of urine samples for up to 3 months in this fashion does not affect the stability of 6-ketoPGF₁α. Heart rate and mean arterial pressure were recorded at the end of each urine collection period.

Serum and urine sodium and potassium were determined using an IL 143 Flame Photometer, creatinine by the alkaline picrate method (Programachem 1040 Autanalyzer), and PAH by the Bratton and Marshal method. Plasma renin activity (PRA) was measured in duplicate using the Angiotensin I Immunotrace kit (Squibb, Princeton, New Jersey).

We measured urine 6-ketoPGF₁α using a previously described radioimmunoassay technique. Briefly, about 1000 cpm 3H-6ketoPGF₁α (100 Ci/mM; New England Nuclear, Boston, Massachusetts) was added to 5 ml urine. The sample was brought to pH 7.0–7.5 and washed with 15 ml hexane. The aqueous layer was titrated to pH 3.5 with concentrated formic acid, then...
extracted with two 15 ml aliquots of ethyl acetate (Burdick Jackson, Muskegon, Michigan). The organic layer was evaporated to dryness under nitrogen and reconstituted in 1 ml of a solvent system: chloroform:heptane:ethanol:acetic acid 100:100:10:2. The sample was placed on a 10 x 130 mm Sephadex LH-20 (Pharmacia Fine Chemicals Ltd., Piscataway, New Jersey) column and eluted with 10 ml of the above solvent system, then successively with 9 ml of chloroform:heptane:acetic acid, 100:100:2:20 and 20 ml of 100:100:30:2. This final fraction, containing the 6-ketoPGF\_\text{\textsubscript{1a}}, was evaporated to dryness under nitrogen and reconstituted in 1.0 ml of a Tris-gelatin buffer. An aliquot was counted for recovery of tritium sample was placed on a 10 x 130 mm Sephadex LH-20 (Pharmacia Fine Chemicals Ltd., Piscataway, New Jersey) column and eluted with 10 ml of the above solvent system, then successively with 9 ml of chloroform:heptane:acetic acid, 100:100:2:20 and 20 ml of 100:100:30:2. This final fraction, containing the 6-ketoPGF\_\text{\textsubscript{1a}}, was evaporated to dryness under nitrogen and reconstituted in 1.0 ml of a Tris-gelatin buffer. An aliquot was counted for recovery of tritium 

To verify the extraction, chromatography, and assay procedures, authentic 6-ketoPGF\_\text{\textsubscript{1a}} (0.13 to 1.95 ng/ml) was dissolved in 30 ml saline for injection for administration to the renal artery. Renal blood flow (RBF) was continuously monitored with appropriately sized magnetic flow probe placed around the renal artery. Catheter placement was verified at the end of each experiment. PAH and creatinine infusion, volume loading and replacement, and equilibration time were similar to the furosemide group.

Secretin (Boots, Nottingham, England) 100 units, was dissolved in 30 ml saline for injection for administration via the renal artery catheter with a Harvard Infusion pump.

The protocol consisted of 17 consecutive 15-minute urine collection periods. Periods 1 through 4 were controls during which saline was infused slowly intravenously. At the end of Period 4, secretin infusion was begun; Periods 5 and 6 were "low" dose, 12.3 ± 2.0 mU/kg/min; Periods 7 and 8 were "medium" dose, 30.3 ± 4.9 mU/kg/min; Periods 9 and 10 were "high" dose, 60.7 ± 10.1 mU/kg/min. Periods 11 and 12 were also controls during which saline was infused. The high dose was again infused during Periods 13, 14, and 15. Indomethacin 10 mg/kg was given intravenously over 5 minutes following Period 14. Urine samples were collected from the left ureter only and handled and analyzed similarly to the furosemide and control studies. MAP, HR, and RBF were recorded at the end of each period. Blood samples, collected at the midpoints of Periods 2, 4, 6, 8, 10, 12, 14 and 16, were stored and analyzed similarly to the furosemide and control groups.

The data refer to only the secretin-infused kidney and are expressed as means ± SEM. A one-way analysis of variance using a TI-59 programmable calculator (Texas Instruments, Dallas, Texas) was used to assess the effects of secretin compared to control values.

Results

Response to Furosemide

Tables 1 and 2 show the responses of furosemide-treated and vehicle-treated (control) dogs. The mean values and SEM for each parameter are given for the 15-minute collection periods immediately before and after each dose of furosemide or vehicle. Not shown are the hematocrit, plasma sodium concentration, and plasma potassium concentration, which remained unchanged in both groups.

When the responses to each dose of furosemide were compared to those to vehicle (control dogs), increases in urine volume (V), sodium excretion (UNaV), and potassium excretion (UKV) were seen at the 0.1 mg/kg and 1.0 mg/kg dosages. Indeed, even the lowest furosemide dose (0.01 mg/kg) increased UNaV. Neither of the two lower doses changed 6-ketoPGF\_\text{\textsubscript{1a}} excretion (U6kV), PAH clearance (CPAH), creatinine clearance (CCr), or plasma renin activity (PRA). In contrast, the highest dose (1.0 mg/kg) resulted in increased U6kV, CPAH, and PRA. CCr was not significantly increased compared to the response in control dogs. Mean arterial pressure and heart rate were not different in the two groups.

In the furosemide-treated group, V, UNaV, UKV, and U6kV returned to or below control levels by 1 hour after each of the two lower doses of furosemide. PRA,
however, increased throughout the experiment. Prior to the 1.0 mg/kg dose, PRA was 5.4 ± 2.2 ng/ml/hr compared with 3.5 ± 1.1 ng/ml/hr (p < 0.05) during the first control period.

Figure 1 depicts the time course of the changes in V, UNaV, CPAH, U6kV, and PRA after the 1.0 mg/kg dose of furosemide. While all measurements increased during the 15 minutes immediately following furosemide injection, U6kV and CPAH returned to pretreatment values during the next 15 minutes while UNaV and V actually increased during this time and remained elevated one hour later.

Figure 2 shows the mean excretion of 6-ketoPGF1α for the furosemide group compared to the mean CPAH for all 16 experimental periods prior to indomethacin. A significant correlation existed between U6kV and PRA.

### Table 1. Responses to Furosemide in Six Dogs

<table>
<thead>
<tr>
<th>Response</th>
<th>0</th>
<th>0.01 mg/kg</th>
<th>0.1 mg/kg</th>
<th>1.0 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>V/ml/15 min</td>
<td>18 ± 9</td>
<td>15 ± 7</td>
<td>25 ± 9</td>
<td>29 ± 10</td>
</tr>
<tr>
<td>UNaV</td>
<td>2.8 ± 1.8</td>
<td>1.9 ± 2.6</td>
<td>1.3 ± 0.3</td>
<td>7.0 ± 1.7</td>
</tr>
<tr>
<td>UKV</td>
<td>0.6 ± 0.7</td>
<td>1.0 ± 1.1</td>
<td>0.9 ± 0.2</td>
<td>1.9 ± 0.4</td>
</tr>
<tr>
<td>CPAH</td>
<td>1.0 ± 1.0</td>
<td>1.1 ± 1.1</td>
<td>1.1 ± 1.1</td>
<td>1.1 ± 1.1</td>
</tr>
<tr>
<td>CCr</td>
<td>41 ± 30</td>
<td>40 ± 30</td>
<td>45 ± 30</td>
<td>49 ± 30</td>
</tr>
<tr>
<td>U6kV</td>
<td>30.3 ± 30.3</td>
<td>49.7 ± 48.7</td>
<td>50.1 ± 46.2</td>
<td>56.0 ± 41.7</td>
</tr>
<tr>
<td>MAP</td>
<td>124 ± 124</td>
<td>122 ± 122</td>
<td>129 ± 129</td>
<td>131 ± 131</td>
</tr>
<tr>
<td>HR</td>
<td>152 ± 152</td>
<td>146 ± 146</td>
<td>148 ± 148</td>
<td>148 ± 148</td>
</tr>
<tr>
<td>PRA</td>
<td>3.5 ± 3.5</td>
<td>5.1 ± 5.1</td>
<td>1.1 ± 1.1</td>
<td>6.9 ± 6.9</td>
</tr>
</tbody>
</table>

Values are means ± SEM for periods immediately before (B) and after (A) doses of vehicle or furosemide and for the first 15-minute period after indomethacin injection (Indo). V = urine volume; UNaV = sodium excretion; UKV = potassium excretion; CPAH = p-aminohippuric acid excretion; CCr = creatinine clearance; U6kV = 6-ketoPGF1α excretion; MAP = mean arterial pressure; HR = heart rate; PRA = plasma renin activity.

* Different from comparable period in control dogs; p < 0.05.

### Table 2. Responses to Vehicle in Three Control Dogs

<table>
<thead>
<tr>
<th>Response</th>
<th>Dose 1</th>
<th>Dose 2</th>
<th>Dose 3</th>
<th>Dose 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>V/ml/15 min</td>
<td>61 ± 20</td>
<td>46 ± 21</td>
<td>67 ± 21</td>
<td>64 ± 20</td>
</tr>
<tr>
<td>UNaV</td>
<td>2.3 ± 0.8</td>
<td>2.2 ± 0.6</td>
<td>1.2 ± 0.6</td>
<td>1.3 ± 0.6</td>
</tr>
<tr>
<td>UKV</td>
<td>1.3 ± 1.0</td>
<td>1.4 ± 1.0</td>
<td>1.2 ± 1.0</td>
<td>1.2 ± 1.0</td>
</tr>
<tr>
<td>CPAH</td>
<td>43 ± 19</td>
<td>32 ± 13</td>
<td>39 ± 13</td>
<td>35 ± 13</td>
</tr>
<tr>
<td>CCr</td>
<td>56 ± 35</td>
<td>55 ± 24</td>
<td>55 ± 24</td>
<td>55 ± 24</td>
</tr>
<tr>
<td>U6kV</td>
<td>159 ± 27</td>
<td>135 ± 27</td>
<td>159 ± 27</td>
<td>148 ± 27</td>
</tr>
<tr>
<td>MAP</td>
<td>108 ± 108</td>
<td>105 ± 105</td>
<td>114 ± 114</td>
<td>119 ± 119</td>
</tr>
<tr>
<td>HR</td>
<td>134 ± 134</td>
<td>132 ± 132</td>
<td>126 ± 126</td>
<td>127 ± 127</td>
</tr>
<tr>
<td>PRA</td>
<td>2.6 ± 1.8</td>
<td>2.0 ± 1.8</td>
<td>2.0 ± 1.8</td>
<td>1.5 ± 1.5</td>
</tr>
</tbody>
</table>

Values are means ± SEM for periods immediately before (B) and after (A) four consecutive doses on vehicle and indomethacin (Indo). Abbreviations as for Table 1.
CPAH \( (r = 0.81, p < 0.001) \). This correlation also existed in each individual dog (range of \( r \) values = 0.77–0.97, not shown). There was no correlation between U6kV and urinary volume \( (r = 0.10) \) or between U6kV and UNaV \( (r = 0.02) \). Finally, UNaV and V were highly correlated \( (r = 0.98, p < 0.001) \). Changes in U6kV in the control group were small and showed no correlation with other parameters.

As mentioned above, and depicted in table 1, PRA tended to increase throughout the experiment in the furosemide-treated group. There was no correlation between U6kV and absolute PRA \( (r = -0.38) \). Presumably, once released by furosemide, PRA decreases more slowly than U6kV. For that reason we examined the relation between the percent increase of PRA to the percent increase of U6kV in each dog before and after each dose of furosemide. The Spearman rank correlation (used because changes in U6kV showed greater range than changes in PRA) was significant \( (r = 0.59, n = 18, p < 0.05) \).

Indomethacin decreased U6kV from 28.6 ± 14.6 to 11.6 ± 6.1 ng/15 min within 15 minutes, a 59% reduction in the furosemide-treated dogs. A similar reduction \( (19.1 ± 7.5 \text{ to } 9.8 ± 2.3 \text{ ng/15 min}; 49\%) \) occurred in the control dogs. No significant effect on other variables was noted, although V, UNaV, CPAH, and CCr all tended to decrease in the dogs treated with furosemide prior to indomethacin.

**Response to Secretin**

In the above experiments we found that renal blood flow and urinary 6-ketoPGF is excretion were highly correlated in dogs treated with furosemide. This result could obtain if PAH and 6-ketoPGF is are handled similarly by the kidney; i.e., the increase in urinary 6-ketoPGF is was secondary to increased renal blood flow. If this were the case, increasing renal blood flow by an alternate agent should increase urinary 6-ketoPGF is. To test this hypothesis, we increased renal blood flow by infusing secretin, since its renal vasodilating effects had been shown to be independent of the prostaglandins.12

Table 3 shows the responses obtained during the final 15 minutes of each 30-minute secretin or saline vehicle infusion. Values are those from the left kidney only. Secretin increased RBF and CPAH in a dose-related fashion \( (p < 0.05) \) but U6kV did not change. MAP, HR, V, UNaV, CCr, hematocrit, serum sodium, and serum or urinary potassium were not changed by secretin. Indomethacin reduced U6kV from 14 ± 1 ng/15 min to 5 ± 3 ng/15 min but did not affect the secretin-induced increases in RBF and CPAH.

There was no correlation between U6kV and RBF, CPAH, CCr, V, or UNaV for the group as a whole or for individual dogs. There was a significant correlation between RBF and CPAH, \( r = 0.68 (p < 0.01) \).

**Discussion**

PGI2 is a major prostaglandin of the renal cortex; thus, the present studies were designed to determine if it might mediate the cortical actions of furosemide, i.e., vasodilation and renin release. We found that furosemide 1.0 mg/kg increased urinary excretion of the PGI2 hydrolysis product 6-ketoPGF is concurrently with increasing CPAH and PRA. The time course of increases in U6kV and CPAH were identical: both had returned to baseline 30 minutes after intravenous injection. In contrast, the natriuresis produced by this dose continued for at least 1 hour. Lower doses of furosemide (0.01 and 0.1 mg/kg) produced natriuresis but...
did not change U6kV, CPAH, or PRA. Thus, this study demonstrates for the first time the dose-dependent nature of the effects of furosemide on PG synthesis and renal blood flow. Because we have clearly shown that renal PGI₂ cannot mediate furosemide-induced natriuresis, the PG releasing action of furosemide is probably not prostaglandin mediated, in contrast to increases in PRA and renal blood flow. Previous studies attempting to demonstrate dependence of furosemide-induced natriuresis on PG production using PG synthesis inhibitors have produced conflicting results, perhaps because these agents can have varying effects on sodium excretion or can reduce the delivery of furosemide to its tubular site of action.

The correlation between CPAH and U6kV in the furosemide-treated dogs (fig. 2) raised the possibility that the increase in U6kV was secondary to increased renal blood flow. This would be particularly true if 6-ketoPGF₁₀ was excreted by the kidney, so that its urinary excretion rate increased when renal blood flow increased. To eliminate this possibility, we increased renal blood flow using secretin because it had been shown to act independently of PGs. Some agents classically used for increasing renal blood flow, such as acetylcholine and bradykinin, were unsuitable because they act, in part through PGs. Infusing secretin into the renal artery increased RBF of that kidney by 63% and CPAH by 48%. Although the increase in CPAH is less than that achieved by furosemide 1.0 mg/kg (15%), we believe that our assay could detect a comparable increase in 6-ketoPGF₁₀. In fact, there was no increase. This, plus the lack of correlation of CPAH or RBF with U6kV in the secretin-infused dogs (individually or as a group) would tend to exclude the hypothesis that furosemide-induced increases in U6kV are secondary to increases in RBF. Rather, the observations are consistent with the notion that furosemide stimulates renal PGI₂ production, which increases RBF, and is excreted as 6-ketoPGF₁₀. However, it must be admitted that these data do not prove this sequence of events or that other PGs are not involved in furosemide-induced vasodilation and renin release. Such proof must await the development of specific PGI₂ synthesis inhibitors or antagonists.

That furosemide-stimulated PG synthesis is responsible in part for increasing renal renin release is supported by the observation that indomethacin blunts this effect of furosemide. In our studies, the rank correlation for the change in U6kV vs change in PRA after furosemide was statistically significant; thus a component of the furosemide induced renal renin release may be via stimulation of renal PGI₂ synthesis. However, furosemide can also stimulate renin release through additional mechanisms not mediated by renal PGI₂.

Urinary 6-ketoPGF₁₀ may more accurately reflect the PG releasing action of furosemide than urinary PGE₂ excretion. Factors such as urinary volume and sodium excretion have been reported to affect PGE₂ excretion, but, in contrast, in our experiments these variables did not correlate with U6kV. However, while it is generally recognized that urine PGE₂ is mostly of renal origin, no comparable data exist for 6-ketoPGF₁₀. Indeed, it is unknown whether 6-ketoPGF₁₀...
circulates in blood. While plasma concentrations of up to 160 pg/ml have been reported in humans, none was detectable in nonpregnant dogs. More recent studies suggest that levels in humans are also very low. In any event, intravenous infusion of labelled PGI₂ or 6-ketoPGF₁α in man or monkey, leads to the appearance of less than 15% of injected material as urine 6-ketoPGF₁α. Thus, the predominant portion of urinary 6-ketoPGF₁α like PGE₂, would appear to be of renal origin under basal conditions, and it seems likely that the furosemide-induced increase in U6kV reflects increased induced renal PGI₂ synthesis.

In summary, we have shown that furosemide, at doses higher than those required for natriuresis, increases urine excretion of the PGI₂, hydrolysis product 6-ketoPGF₁α. This increased excretion appears to reflect PGI₂ action at the renal cortex, as evidenced by the simultaneous increase in CPAH and PRA. Natriuresis due to furosemide appears independent of PGI₂ release. The relative role of other cyclooxygenase products, such as PGE₂, in vasodilation, renin release, and natriuresis is unknown but it appears that urinary excretion of 6-ketoPGF₁α accurately reflects the actions of furosemide other than natriuresis.

Acknowledgments

We thank Marsha Black, Harold Thibodeaux, and Stephanie Schwade for expert technical assistance, John Morrison for measurements of PAH concentrations; and Juanita Pike and Margaret Wilson for excellent secretarial assistance.

References

URINARY 6-KETO PROSTAGLANDIN F\textsubscript{1α} /Wilson et al.


Furosemide increases urine 6-keto-prostaglandin F1 alpha. Relation to natriuresis, vasodilation, and renin release.
T W Wilson, C B Loadholt, P J Privitera and P V Halushka

Hypertension. 1982;4:634-641
doi: 10.1161/01.HYP.4.5.634

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1982 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/4/5/634.citation