Role of Prostaglandins in Mediating the Renal Effects of Atrial Natriuretic Factor

F. JAVIER SALAZAR, RODNEY BOLTERMAN, MARY J. FIKSEN-OLSEN, THOMAS QUESADA, AND J. CARLOS ROMERO

SUMMARY The natriuretic response to the intrarenal administration of atrial natriuretic factor (ANF) is accompanied by an increase in the synthesis of prostaglandins and by a redistribution of renal blood flow from the superficial to the deep cortex. This study was undertaken to define whether prostaglandins mediate the ANF-induced redistribution of renal blood flow and if prostaglandins and renal blood flow redistribution contribute to the natriuretic actions of ANF. In anesthetized dogs, the intrarenal administration of indomethacin (10 μg/kg/min) or the intravenous administration of meclofenamate (5 mg/kg) completely prevented the sixfold and twofold increments in urinary prostaglandin E_2 and 6-keto-prostaglandin F_1α excretion, respectively; it also abolished the redistribution of renal blood flow to the deep cortex. However, ANF induced a similar natriuresis before (from 53 ± 17 to 281 ± 48 μEq/min) and after (from 45 ± 13 to 273 ± 60 μEq/min) the administration of prostaglandin synthesis inhibitors. It is concluded that the ANF-induced redistribution of renal blood flow to the deep cortex is prostaglandin-mediated but that neither redistribution nor increased prostaglandin synthesis is an important mediator of ANF's natriuretic action. (Hypertension 12: 274-278, 1988)

KEY WORDS • atrial natriuretic factor • intrarenal blood flow distribution • prostaglandin synthesis inhibitors • sodium excretion

We have recently found that the natriuretic response to the intrarenal infusion of atrial natriuretic factor (ANF) at doses that do not alter mean arterial pressure (MAP), renal blood flow (RBF), or glomerular filtration rate (GFR) is accompanied by a significant increase in urinary excretion of prostaglandins and by a redistribution of RBF from the superficial to the juxtamedullary cortex. Previous studies have shown that enhanced prostaglandin synthesis or selective prostaglandin infusion increases juxtamedullary cortical flow. Others have shown that blockade of prostaglandin synthesis decreases juxtamedullary cortical flow more than superficial cortical flow. Increases in medullary flow have been shown to produce natriuresis, as has increased prostaglandin synthesis. We therefore attempted to define 1) the role of prostaglandins in the ANF-induced redistribution of RBF to the juxtamedullary cortex, 2) the role of prostaglandins in ANF-induced natriuresis, and 3) the role of the redistribution of RBF in natriuresis. This was done in anesthetized dogs by evaluating the effects of ANF on RBF distribution and on urinary sodium excretion before and after the blockade of prostaglandin synthesis with indomethacin or meclofenamate.

Materials and Methods

Experiments were performed on mongrel dogs of either sex (weight, 16–20 kg) maintained on a standard laboratory diet with free access to water. Food was withheld on the day of the experiment. Dogs were anesthetized with sodium pentobarbital (30 mg/kg i.v.) and ventilated artificially with a Harvard respirator (South Natick, MA, USA). The minute volume ventilation was selected by reference to the nomogram of Kleinman and Radford. Catheters were placed in the femoral artery for measurements of MAP and in the femoral vein for infusion of additional anesthesia (0.8 mg/min) and inulin. Inulin was dissolved in isotonic saline and
Infusion was begun. And again 30 minutes after the second ANF, the infusion was stopped, 30 minutes was allowed for parameters to return to control. At this time, an additional 15-minute clearance was obtained. Subsequently, an intravenous bolus of meclofenamate (5 mg/kg; n = 3) was administered or an intrarenal infusion of indomethacin (10 μg/kg/min; n = 3) was begun and continued until the end of the experiment. A 15-minute clearance was obtained 30 minutes after either the meclofenamate bolus or the start of the indomethacin infusion, after which a second 30-minute intrarenal ANF infusion (0.05 μg/kg/min) was started. As in the control period, two clearances of 5 minutes each were obtained at 20 and 25 minutes during the ANF infusion; after the infusion was stopped, 30 minutes was allowed for parameters to return to control. At this time, an additional 15-minute clearance was obtained. Subsequently, an intravenous bolus of meclofenamate (5 mg/kg; n = 3) was administered or an intrarenal infusion of indomethacin (10 μg/kg/min; n = 3) was begun and continued until the end of the experiment. A 15-minute clearance was obtained 30 minutes after either the meclofenamate bolus or the start of the indomethacin infusion, after which a second 30-minute intrarenal ANF infusion (0.05 μg/kg/min) was started. As in the control period, two clearances of 5 minutes each were obtained at 20 and 25 minutes during the ANF infusion.

The intrarenal cortical distribution of RBF was measured using 15-μm microspheres labeled with 153Gd, 57Co, 111In, 99mTc (DuPont–New England Nuclear). The sequence of nuclide injection was randomized. The stock solution of microspheres came suspended in 10% dextran with 0.01% polysorbate 80 (Tweeen 80) surfactant added to prevent aggregation of spheres. Before injection the stock solution of spheres was mechanically agitated and ultrasonicated. Approximated 7 x 10^5 spheres were withdrawn from the stock solution and suspended in 1 ml of 37 °C saline for injection into the left ventricular catheter. The microspheres were immediately flushed into the left ventricle with 10 ml of saline warmed to 37 °C. The stopcock of the left ventricular catheter was replaced after injection of each nuclide species to minimize retention of microspheres in the injection system. Microspheres were injected during the control period, 30 minutes after beginning the infusion of ANF, 45 minutes after the meclofenamate bolus or indomethacin infusion was started, and again 30 minutes after the second ANF infusion was begun.

At the end of the experiment, the kidneys were removed and renal cortices were divided into four equal zones that were designated Zones 1 through 4, going from outer to inner cortex, respectively. The medullary and renal poles, which could not be divided accurately into zones, were treated as separate zones so that total RBF could be evaluated. The tissue for each zone was weighed and placed in polypropylene counting tubes. Radioactivity was measured using an LKB 1282 CompuGamma (Turku, Finland). Isotope separation was achieved as previously described. Fractional cortical blood flow was calculated by taking the radiation for a given cortical zone divided by total radiation for the four zones multiplied by 100.

Group 2
The experimental protocol in Group 2 (n = 4) was similar to that for Group 1 except that isotonic saline was administered instead of indomethacin or meclofenamate.

Analytical Methods
GFR was measured by the clearance of inulin. Inulin concentrations were analyzed by the anthrone method. Concentrations of sodium and potassium were measured using ion-selective electrodes (Beckman E2A analyzer, Brea, CA, USA). Urinary prostaglandins E2 (PGE2) and 6-keto-F1α (6-keto-PGF1α) were measured by radioimmunoassay according to the method previously described. Analysis of variance for randomized block design was used to test the null hypothesis, that there were no changes with each maneuver. The Newman-Keuls test was used to determine significant differences between maneuvers. Results are presented as means ± SE.

Results
Group 1
Table 1 shows that the intrarenal infusion of ANF produced a significant increase in urine volume and urinary sodium and potassium excretion without altering significantly MAP, RBF, or GFR. The increases in urine volume and sodium and potassium excretion induced by ANF returned to control values during the recovery period. Table 1 also shows that the administration of meclofenamate or indomethacin produced an average increase of 8 mm Hg in MAP (p < 0.05) and an average decrease of 32 ml/min in RBF as compared with values in the recovery period. These changes were not accompanied by any significant alteration in GFR. Sodium excretion decreased, in five of the six dogs, when compared with that in the recovery period; however, this decrease did not achieve statistical significance. The administration of ANF in the presence of prostaglandin inhibitors produced an elevation in urinary sodium and potassium excretion (see Table 1) comparable to that produced by ANF in the absence of the prosta-
TABLE 1. Effects of Intrarenal Infusion of ANF With and Without the Simultaneous Administration of Indomethacin or Meclofenamate

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>ANF</th>
<th>Recovery</th>
<th>Indom or Meclof</th>
<th>Indom or Meclof + ANF</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>118 ± 7</td>
<td>115 ± 7</td>
<td>120 ± 5</td>
<td>128 ± 6*</td>
<td>126 ± 7</td>
</tr>
<tr>
<td>RBF (ml/min)</td>
<td>193 ± 20</td>
<td>195 ± 22</td>
<td>190 ± 22</td>
<td>158 ± 23*</td>
<td>177 ± 22†</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td>32 ± 3</td>
<td>33 ± 3</td>
<td>31 ± 3</td>
<td>32 ± 3</td>
<td>32 ± 4</td>
</tr>
<tr>
<td>(U_{Na}V) (μEq/min)</td>
<td>53 ± 17</td>
<td>281 ± 48†</td>
<td>66 ± 8</td>
<td>45 ± 13</td>
<td>273 ± 60†</td>
</tr>
<tr>
<td>(U_KV) (μEq/min)</td>
<td>26 ± 4</td>
<td>45 ± 8‡</td>
<td>26 ± 4</td>
<td>29 ± 7</td>
<td>49 ± 11†</td>
</tr>
<tr>
<td>V (ml/min)</td>
<td>0.4 ± 0.1</td>
<td>2 ± 0.45</td>
<td>0.4 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>2.7 ± 0.5‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. Indom = indomethacin; Meclof = meclofenamate; RBF = renal blood flow; GFR = glomerular filtration rate; \(U_{Na}V\) = urinary sodium excretion; \(U_KV\) = urinary potassium excretion; V = urine volume.

*\(p < 0.05\), compared with recovery values.
†\(p < 0.05\), ‡\(p < 0.1\), compared with values for indomethacin or meclofenamate alone.
§\(p < 0.05\), ||\(p < 0.1\), compared with control values.

The fractional distribution of cortical blood flow during the intrarenal infusion of ANF with and without inhibition of prostaglandin synthesis is shown in Figure 1. During the infusion of ANF alone, the percentage of cortical blood flow that perfused the deep cortical areas (Zones 3 and 4) was significantly increased \((p < 0.05)\), whereas the percentage of cortical blood flow that perfused the superficial (Zone 1) was significantly decreased \((p < 0.05)\). Inhibition of prostaglandin synthesis resulted in a significant decrease in the percentage of cortical flow to Zone 4, while the percentages of cortical flow in Zones 1, 2, and 3 were similar to that found in the control period. The redistribution of RBF to the deep cortex observed with the first infusion of ANF did not occur when ANF was administered after the inhibition of prostaglandin synthesis.

Urinary excretion of PGE\(_2\) and 6-keto-PGF\(_{1α}\) are shown in Figure 2. The intrarenal administration of ANF induced a significant increase in the urinary excretory rate of PGE\(_2\) (from 553 ± 363 to 1300 ± 376 pg/min) and 6-keto-PGF\(_{1α}\) (from 1560 ± 545 to 2171 ± 271 pg/min), which returned to control levels after discontinuation of the ANF infusion. During prostaglandin inhibition, the urinary excretory rates of PGE\(_2\) and 6-keto-PGF\(_{1α}\) decreased below control levels and administration of ANF failed to alter the excretory rate of either PGE\(_2\) or 6-keto-PGF\(_{1α}\).

Group 2

The results obtained in this group, which underwent a protocol similar to that used for Group 1 but without the infusion of indomethacin or meclofenamate, are shown in Table 2. Both infusions of ANF induced comparable increases in urine volume and urinary sodium and potassium excretion, along with comparable redistribution of RBF from the outer (Zone 1) to the inner cortex (Zones 3 and 4).

Discussion

Increasing the synthesis of renal prostaglandins by the administration of arachidonic acid, prostaglandin synthesis precursor, or selective prostaglandin infusions has been shown to produce a marked redistribution of RBF to the juxtamedullary cortex.\(^2-4\) ANF has been reported to induce a similar redistribution of RBF to the juxtamedullary cortex\(^1,15\) along with an increase in urine prostaglandin excretion.\(^1\) Therefore, we examined the distribution of RBF in the absence and presence of...
prostaglandin inhibitors. We observed that the administration of prostaglandin inhibitors produced a decrease in basal RBF that was accompanied by a small decrease in juxtamedullary flow. Others have also reported similar changes in basal RBF and RBF distribution.\(^5\)\(^6\) ANF administration in the presence of prostaglandin inhibition failed to increase the fractional juxtamedullary flow, even in the presence of a small increase in RBF. Others have suggested that a redistribution of blood flow to the juxtamedullary cortex during renal vasodilation may allow an increased number of microspheres to gain access to deep cortical afferent arterioles.\(^16\) However, under our experimental conditions, there was no increase in RBF during the control ANF infusion, which resulted in a redistribution toward the juxtamedullary cortex, and no redistribution was seen with the ANF infusion in the presence of prostaglandin inhibitors when RBF increased \(12\%\). These data suggest that prostaglandins are important in maintaining basal RBF distribution\(^7\) and that prostaglandins are also involved in the ANF-induced redistribution to the juxtamedullary cortex. Furthermore, our data indicate that prostaglandins are important in basal natriuresis, since the inhibition of prostaglandins resulted in a significant decrease in the control levels of urinary sodium excretion. However, the ANF-induced natriuresis was not altered by prostaglandin inhibition. This finding suggests that the increase in prostaglandin excretion seen with the infusion of ANF under control conditions is not mediating the natriuresis. Keele\(^12\) also has shown that the natriuretic effect of ANF is not prostaglandin-mediated.

Others have shown that acetylcholine, a vasodilator, produces a natriuretic effect accompanied by an increase in medullary blood flow\(^8\)\(^9\) and that secretin, a vasodilator, fails to produce natriuresis or increase medullary blood flow.\(^9\) The present study demonstrated that the ANF-induced natriuresis is not dependent on a redistribution of RBF to the juxtamedullary cortex.

The results from the administration of meclofenamate and indomethacin were pooled since the changes were similar in the parameters measured. This suggests that the results are due to prostaglandin synthesis inhibition rather than to some specific effect of either inhibitor. The measures of urinary PGE\(_2\) and 6-keto-PGF\(_{1alpha}\) during prostaglandin blockade indicate that the inhibitors were effective in blocking prostaglandin synthesis.

In summary, the results of this study indicate that the ANF-induced redistribution of RBF to the juxtamedullary cortex, as measured by radiolabeled microspheres, is mediated by the increase in prostaglandin synthesis. However, neither the redistribution of RBF nor the increased synthesis of prostaglandins appears to be an important mediator of the ANF-induced natriuresis.

**Acknowledgments**

The secretarial assistance of Maria J. Salazar and Paulette Peterson is gratefully acknowledged.

**References**


**Table 2. Effects of Intrarenal Infusion of ANF**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>ANF</th>
<th>Recovery</th>
<th>ANF 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>117±8</td>
<td>114±10</td>
<td>121±9</td>
<td>120±8</td>
</tr>
<tr>
<td>RBF (ml/min)</td>
<td>190±10</td>
<td>198±10</td>
<td>181±7</td>
<td>184±9</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td>30±1</td>
<td>32±1</td>
<td>34±1</td>
<td>34±1</td>
</tr>
<tr>
<td>U(_{\text{Na}})V (µEq/min)</td>
<td>32±16</td>
<td>242±30*</td>
<td>44±9</td>
<td>251±34†</td>
</tr>
<tr>
<td>U(_{\text{K}})V (µEq/min)</td>
<td>18±4</td>
<td>42±3*</td>
<td>20±2</td>
<td>41±4</td>
</tr>
<tr>
<td>V (ml/min)</td>
<td>0.2±0.1</td>
<td>1.7±0.2</td>
<td>0.2±0.1</td>
<td>2.0±0.3</td>
</tr>
</tbody>
</table>

Values are means ± SE. The second infusion of ANF (ANF 2) was performed after the recovery period that followed the first infusion. Abbreviations are the same as for Table 1.

\(^*p < 0.05, \text{ compared with control values.}\)

\(^{†}p < 0.05, \text{ compared with recovery values.}\)
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