Atrial Natriuretic Peptide Suppresses Compensatory Renal Growth in Rats

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Abstract
Atrial natriuretic peptide (ANP) inhibits the growth of a variety of cell types in vitro including mesangial cells. The effects of ANP on the growth of the kidney in vivo were evaluated. A 2-h infusion of 0.2 µg/250 g body wt per minute of ANP suppressed the subsequent uptake of [3H]thymidine into the renal DNA of uninephrectomized but not intact rats. This treatment also depressed the ratio of RNA/DNA in kidneys undergoing compensatory growth. Correlative physiologic studies revealed enhanced GFR in rats with two kidneys infused with ANP, but no increase in the GFR of uninephrectomized rats. It was concluded that ANP may oppose the growth factor(s) mediating compensatory renal growth.

Key Words: Uninephrectomy, DNA synthesis, renal hypertrophy, angiotensin II

Important role in compensatory renal growth (6), and ANP has been shown to block angiotensin II–stimulated proliferation of mesangial cells (7). Therefore, the compiled evidence might be interpreted to suggest that endogenous ANP promotes the physiologic adaptation to a reduction in renal mass but restrains the compensatory growth.

In this study, we elected to evaluate the effect of an acute infusion of ANP on compensatory renal growth in vivo. We selected an acute infusion of ANP to avoid down-regulation of the biologic receptors for the peptide, which might accompany a chronic elevation of plasma ANP levels (8). We also wanted to assess the acute effect of a high dose of ANP on the rate of DNA synthesis within a time frame that has previously been used to assess the effect of a renal mitogen in vivo (9). Concurrent physiologic studies in similarly operated conscious animals were undertaken to assess the effects of the infusion of the same dose of ANP on blood pressure and GFR. We observed that ANP suppresses compensatory renal growth.

Materials and Methods
Animals
Male Sprague-Dawley rats weighing 275 to 350 g were used in all studies. The animals were maintained in clear plastic cages on standard lab chow (LM485, Na = 0.31%; Teklad, Madison, WI) with free access to water. They were housed in the Animal Research Facility of the Tucson VA Medical Center where the ambient temperature is maintained at approximately 25°C with a light:dark cycle of 12:12 h.

Nephrectomy
Animals were anesthetized with im Inovar® (fentanyl-droperidol; 0.03 mL/100 g body wt). The left kidney was exposed via a small flank incision, the renal pedicle was ligated, and the kidney was excised. The sham operation involved exposure and manipulation of the left kidney without removal.

Assessment of Renal Growth
Animals were divided into four groups of six to seven and anesthetized with Inovar® (0.03 mL/100 g body wt). Uninephrectomized animals were studied in April, and animals undergoing the sham operation were studied in June. A femoral venous cannula was
secured and tunneled to exit behind the skull. During the same period of anesthesia, the animal underwent either uninephrectomy or sham nephrectomy. The animals were allowed to recover, and the lines were maintained with heparinized dextrose solution. Forty-eight hours later, one group of sham-operated an uninephrectomized rats received a 2-h infusion of 0.2 μg/250 g body wt per minute of ANP (Atriopeptin III, a 28-amino-acid synthetic product of Peninsul Labs, Belmont, CA) in 0.9% saline delivered at 1.5 mL/100 g body wt per hour via the femoral venous access. Control sham-operated and uninephrectomized groups received an infusion of the vehicle in the same volume. Eighteen hours after the administration of ANP, an infusion of [3H]thymidine (Dupont, NEN Research Products, Boston, MA; 90 to 110 Ci/mmole) was initiated. Each animal received 75 μCi of [3H]thymidine in 1.5 mL of 0.9% saline over 1.5 h. At the end of this period, the animal was euthanized by the administration of an iv injection of Inactin®. Blood was collected, the right kidney was removed and weighed, and a portion was frozen and stored at −80°C. The serum concentrations of [3H]thymidine at the end of the infusion period were assessed by determining the radioactivity of an aliquot of serum. One animal from the vehicle-infused uninephrectomized group had to be excluded because of the finding of extravasation of the infusate around the femoral vein.

Frozen kidney pieces were subsequently homogenized in water to yield a concentration of 50 mg/mL. The homogenate was added to 20% trichloroacetic acid, and the resultant precipitate was heated for 30 min at 90°C in 5% trichloroacetic acid. The total concentration of DNA in the supernatant was measured by the diphenylamine-acetaldehyde colorimetric method (10). The concentration of labeled DNA was determined by liquid scintillation counting. The same supernatant was used for the determination of the total RNA concentration with a colorimetric assay in which pentose is converted to furfural in the presence of hot acid, which then reacts with orcinol to yield a green color (11). A standard curve was prepared with calf liver RNA (Sigma Chemical Co., St. Louis, MO), and interference from DNA was subtracted from each sample on the basis of a separate set of standards prepared with calf thymus DNA (Sigma). The tissue protein concentrations were determined by the method of Lowry et al. (12).

Assessment of Renal Function

GFR was assessed by determining the clearance of labeled inulin after a single injection as previously described and modified for use in the rat (13, 14). Two to 5 days before the clearance measurements, two groups of animals were anesthetized with iminovar® (0.03 mL/100 g body wt). The femoral artery and vein were exposed and cannulated (Tygon; internal diameter, 0.06 inches x outer diameter, 0.13 inches). After the cannulae was secured in the vessel, the tubing was tunneled sc to exit and be secured just behind the skull. The lines were filled with 50% dextrose containing 50% vol/vol heparin (100 U/mL) and capped. The animals were allowed to recover fully, and the lines were maintained by replacing the dextrose and heparin at least every other day. The clearance studies were performed in gently restrained, unanesthetized animals. A total of seven uninephrectomized and five sham-operated animals were studied. Blood pressure was determined via the arterial line (Gould P-23D transducer, SP-1400 monitor) immediately before the injection of inulin. The hematocrit was also determined from an arterial blood sample. [3H]inulin (Dupont, NEN Research Products; 100 to 500 μCi/g) in 0.9% saline was rapidly infused via the venous access. Arterial blood samples were collected 1 min after injection and then every 5 min for 2 h. The plasma disintegrations per minute concentrations were determined by scintillation counting. The GFR was estimated by the formula:

\[
GFR = \frac{\text{Total amount of Indicator injected}}{\text{Area under the disappearance curve}}
\]

The area under the disappearance curve was calculated by computer-aided numerical integration (Datatit; Morroware, Austin, TX).

Inulin clearances were determined before surgery and 48 h after either left nephrectomy or sham left nephrectomy. Immediately after the baseline post-nephrectomy clearances, ANP (0.2 μg/250 g body wt per minute) was infused in 0.9% saline delivered at 1.5 mL/100 g body wt per hour. A final inulin clearance was determined after 2 h of infusion of ANP in both sham-operated and uninephrectomized rats.

Statistics

Paired t tests were used to compare the inulin clearances measured sequentially in the same animal. The other data were evaluated by one-way analysis of variance followed by Fisher’s least significant differences test. Differences between means were considered significant if \( P < 0.05 \) in a two-tailed normal distribution.

RESULTS

The animals used for the growth assays were weighed before surgery and euthanasia 72 h later. Animals undergoing sham nephrectomy were slightly smaller than the uninephrectomized rats at the beginning of the study period. None of the animals regained their preoperative weight, but neither uninephrectomy nor ANP infusion had an independent
significant effect on the amount of body weight lost. However, uninephrectomized animals treated with the ANP infusion lost more body weight than sham-operated animals receiving the vehicle, and uninephrectomized animals treated with the ANP infusion had a lower final body weight than uninephrectomized controls (Table 1).

ANP infusion did not alter the right kidney weight of uninephrectomized rats or the combined weight of the two kidneys in sham-operated animals (Table 1). The mean renal protein contents expressed per microgram of DNA were significantly higher in both uninephrectomized groups compared with sham-operated controls, and ANP did not significantly alter this ratio. The renal RNA/DNA ratio, an index of hypertrophy, was also not influenced by ANP infusion in sham-operated animals. A significant compensatory increase in the renal RNA/DNA ratio was observed in uninephrectomized animals given vehicle compared with sham-operated controls (Table 2). However, uninephrectomized rats given ANP did not have a significantly higher renal RNA/DNA ratio than sham-operated rats given ANP. Uninephrectomized rats treated with ANP had a significantly depressed renal RNA/DNA ratio compared with uninephrectomized rats given vehicle (Table 2).

The mitogenic effect of uninephrectomy was also blunted by the ANP infusion. The specific activity of renal DNA was significantly higher in uninephrectomized controls compared with control sham-operated animals. ANP infusion 18 h earlier significantly depressed the subsequent uptake of [3H]thymidine into renal DNA in uninephrectomized rats (Table 2). The specific activity of DNA in ANP-infused uninephrectomized rats was not significantly different from that in sham-operated animals infused with ANP. There was no effect of ANP infusion on the basal uptake of the labeled DNA precursor in sham-operated animals. The mean serum concentrations of [3H]thymidine at the end of the infusion period were not different between the groups (dpm x 10^4 in 50 μL of serum, sham + vehicle = 25.4 ± 1.6; sham + ANP = 25.5 ± 0.9; UN + vehicle = 22.1 ± 0.6; UN + ANP = 21.5 ± 1.5).

Baseline GFR were identical in the two groups of animals undergoing either uninephrectomy or sham operation (Figure 1). Two days after surgery, the mean GFR was lower in uninephrectomized rats compared with sham-operated controls. However, physiologic compensation to the 50% reduction in renal mass was suggested because the uninephrectomized rats had a mean of about 60% of the baseline GFR of that of intact sham-operated controls. ANP infusion significantly increased GFR in sham-operated rats but did not further increase GFR in the uninephrectomized animals (Figure 1). The systemic mean arterial pressure was reduced by ANP infusion to a similar degree in both intact and uninephrectomized rats (Table 3).

### Table 1. Body and kidney weights of sham-operated and uninephrectomized rats infused with ANP or the saline vehicle

<table>
<thead>
<tr>
<th>Group (N)</th>
<th>Initial Body Wt (g)</th>
<th>Final Body Wt (g)</th>
<th>Loss of Body Wt (g)</th>
<th>Final Kidney Wt (g)</th>
<th>Dry Kidney Wt (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SH + VEH (6)</td>
<td>293 ± 4</td>
<td>281 ± 5</td>
<td>10 ± 3</td>
<td>1.90 ± 0.05</td>
<td>484 ± 7</td>
</tr>
<tr>
<td>SH + ANP (7)</td>
<td>302 ± 3</td>
<td>287 ± 4</td>
<td>17 ± 5</td>
<td>1.98 ± 0.06</td>
<td>498 ± 15</td>
</tr>
<tr>
<td>UN + VEH (5)</td>
<td>322 ± 10</td>
<td>302 ± 4</td>
<td>19 ± 4</td>
<td>1.13 ± 0.04</td>
<td>282 ± 9</td>
</tr>
<tr>
<td>UN + ANP (6)</td>
<td>317 ± 6</td>
<td>290 ± 3</td>
<td>27 ± 3</td>
<td>1.11 ± 0.03</td>
<td>273 ± 7</td>
</tr>
</tbody>
</table>

*All values shown are means ± SE. SH refers to sham-operated rats, and UN refers to those undergoing uninephrectomy. VEH refers to treatment with the saline vehicle and ANP refers to treatment with the ANP infusion.

P < 0.025 versus SH + VEH.

P < 0.025 versus SH + ANP.

P < 0.025 versus UN + VEH.

### Table 2. Renal growth parameters in kidneys of intact and uninephrectomized rats

<table>
<thead>
<tr>
<th>Group (N)</th>
<th>Protein: DNA Ratio</th>
<th>RNA: DNA Ratio</th>
<th>Sp Act DNA (dpm/μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SH + VEH (6)</td>
<td>30.5 ± 0.4</td>
<td>1.77 ± 0.05</td>
<td>32.9 ± 0.8</td>
</tr>
<tr>
<td>SH + ANP (7)</td>
<td>29.9 ± 0.2</td>
<td>1.79 ± 0.04</td>
<td>35.8 ± 4.6</td>
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<tr>
<td>UN + VEH (5)</td>
<td>54.4 ± 4.0</td>
<td>3.39 ± 0.33</td>
<td>67.9 ± 6.2</td>
</tr>
<tr>
<td>UN + ANP (6)</td>
<td>47.3 ± 1.0</td>
<td>2.37 ± 0.32</td>
<td>48.4 ± 4.1</td>
</tr>
</tbody>
</table>

*Values are mean ± SE. SH, sham uninephrectomy; VEH, vehicle.

P < 0.025 versus SH + VEH.

P < 0.025 versus SH + ANP.

P < 0.025 versus UN + VEH.
Figure 1. The bars represent the means ± SE for uninephrectomized (UN) rats (N = 7) and sham-operated (SH) rats (N = 5) before surgery (PRE-UN and PRE-SH), after surgery (UN and SH), and after postoperative ANP infusion (UN + ANP and SH + ANP). *P < 0.05 compared with the preceding bar.

TABLE 3. The effect of ANP on blood pressure in intact and uninephrectomized rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline MAP (mm Hg)</th>
<th>Postoperative MAP (mm Hg)</th>
<th>Post-ANP MAP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SH (5)</td>
<td>122 ± 1</td>
<td>119 ± 4</td>
<td>100 ± 4b</td>
</tr>
<tr>
<td>UN (7)</td>
<td>121 ± 4</td>
<td>114 ± 3</td>
<td>98 ± 2b</td>
</tr>
</tbody>
</table>

MAP: mean arterial pressure.

b P < 0.01 versus baseline and postoperative.

DISCUSSION

Adaptation to a reduction in renal mass includes both physiologic and anatomical responses. The cause and effect relationship between these two categories of biologic events has been questioned for many years, and one longstanding theory states that increased renal work governs growth (15). On the other hand, compensatory hyperplasia has been observed to precede the enhancement of GFR in some studies (16). Furthermore, compensatory changes in renal function and mass can be dissociated under certain experimental conditions (17, 18). Therefore, it is possible that compensatory changes in renal function and mass precipitated by partial nephrectomy are independently regulated.

The results of this study, combined with the work of others, raise the intriguing possibility that ANP participates in both the physiologic and growth response of the remaining kidney to a reduction in renal mass, but in opposite directions. The idea that ANP plays a key role in the physiologic compensation of the remaining kidney was suggested by Valentin and coworkers (1). Excision of the right atrial appendage in rats prevented the significant increases in plasma ANP levels noted in control rats 2 h after uninephrectomy. Right atrial appendectomy also prevented the natriuresis and diuresis that immediately followed uninephrectomy. ANP infusion in the isolated perfused kidney in intact animals causes a sustained increase in GFR, even when systemic arterial pressure is reduced and RBF is unchanged or reduced (19). If the work hypertrophy theory is correct, then ANP infusion should stimulate compensatory renal growth and right atrial appendectomy should diminish it. In this study, we observed a significant increase in GFR of intact, sham-operated animals perfused with ANP. However, the compensatory increase in GFR that followed uninephrectomy was not further increased by the ANP infusion, so that specific testing of the work hypertrophy hypothesis did not apply. One previous in vivo evaluation of the effects of ANP on renal growth revealed an increase in glomerular volume with ANP infusion in rats after 5% nephrectomy. However, there was no effect of ANP on kidney weight, the only other marker of renal growth, described in this experiment (20).

An assessment of compensatory renal growth was not included in the studies where the source of ANP was removed by atrial appendectomy (1), but would also be of interest in further understanding the role of the ANP-induced changes in GFR on compensatory renal growth.

The fact that ANP inhibits the growth of the kidney independent of its effects on GFR is suggested by the results of in vitro experiments (21). The results of this study provide novel in vivo evidence that a pharmacologic dose of ANP suppresses compensatory renal growth. Compensatory renal growth was clearly in progress in all of the uninephrectomized animals included in these studies, as evidenced by the similar kidney weights. However, on the second postnephrectomy day, a 2-h infusion of ANP significantly suppressed the uptake of [3H]thymidine into renal DNA measured 18 h later. The decision to infuse the ANP 2 days after uninephrectomy was based on the fact that this is the time when the peak mitogenic activity has been observed in compensatory renal growth (22). The rationale for assessing the effect of the ANP infusion on the rate of uptake of thymidine 18 h later was based on previous observations in vitro and in vivo. Quiescent kidney cells in culture have been shown to have the maximum uptake of thymidine 18 h after a mitogenic stimulus (23, 24). Previous work has shown that injection of the known renal mitogen thyroxine significantly increases the in vitro uptake of [3H]thymidine by the kidney 18 h later (9). The results of this study confirm a significant effect of uninephrectomy on the rate of renal uptake of thymidine 3 days after uninephrectomy and confirm that an ANP infusion on the second day significantly attenuates this mitogenic effect.

Uninephrectomized rats infused with ANP also had
a lower RNA/DNA ratio in the remaining kidney compared with uninephrectomized controls. This result suggests interference with the hypertrophic response to uninephrectomy. The protein/DNA ratio tended to be lower as well in uninephrectomized rats infused with ANP. The lack of significant depression of ANP on the protein/DNA ratio may be related to increased variability of this marker hypertrophy imposed by the amount of blood trapped in the kidney at the time of collection (25). It is also possible that the short 2-h exposure to ANP 18 h before euthanasia was sufficient to interfere with RNA but not protein synthesis or degradation.

One confounding variable that requires consideration is the observation that rats infused with ANP tended to lose more body weight than controls. If ANP suppressed food intake, then suppression of compensatory renal growth might reflect a simple difference in caloric and protein intake. The design of the experiment allowed rats to eat ad libitum for 48 h after surgery before the infusion of ANP or saline. Although the animals were not pair fed, there is no reason to expect that the groups, which were otherwise untreated for those 2 days, would have consumed different amounts of food. Eighteen hours after the infusion of ANP or saline, the animals were euthanized. If differences in food intake were to explain the effect of ANP on compensatory renal growth, it would have most likely had to occur during that 18-h period between the infusion of the peptide and euthanasia. Other studies have shown that total deprivation of food for up to 24 h does not suppress the mitotic count in uninephrectomized rats compared with uninephrectomized controls eating ad libitum, but inhibition of the number of mitoses was observed after 32 h of starvation (26). Therefore, it is reasonable to suggest that the observed effect of ANP on DNA synthesis 18 h later was unrelated to food intake during that same interval. Finally, we are unaware of any reports of suppression of food intake in animals infused with ANP.

The alternative explanation for the lower final body weights in animals infused with ANP after uninephrectomy is that the peptide prompted an acute natriuresis and diuresis and that these losses were not completely recaptured at the time the animals were weighed. Martin and coworkers found that the infusion of approximately one-half of the dose of ANP used in this study to rats caused a fourfold increase in urine flow rate and a fivefold increase in urine sodium excretion within 2 h (27). Therefore, it is reasonable to assume that the animals used in this study had similar losses of salt and water, which may explain their tendency for lower final body weights.

The dose of ANP administered would be expected to raise ANP levels far above physiologic ranges (28), so that the suppression of compensatory growth observed in this study was a pharmacologic effect. However, this pharmacologic effect may be relevant to the physiologic role of ANP as a modulator of compensatory renal growth. Indirect evidence of ANP action in the physiologic context is suggested from the observed changes in cyclic nucleotide metabolism during compensatory renal growth. Schlondorff and Weber found a 200% increase in renal cGMP beginning 1 h after uninephrectomy (2). Valentin and coworkers confirmed enhanced urinary cGMP excretion postuninephrectomy, which could be prevented by right atrial appendectomy (1). Therefore, it is possible that this enhanced accumulation of cGMP during compensatory renal growth reflects ANP action not only on renal physiology but also as a modulator of growth. The idea that cGMP is the mediator of the inhibitory effect of ANP on cell growth has been recently considered and is controversial (20). However, Wolf and coworkers demonstrated that, like ANP, a cGMP analog inhibited angiotensin II–induced proliferation of mesangial cells (7).

It would be reasonable to consider that the suppression of compensatory growth by a pharmacologic dose of ANP may have relevance to a physiologic effect if the responses of plasma ANP and its secondary messenger cGMP to uninephrectomy are submaximal, allowing ANP to only dampen growth stimulation by other factors. Hence, raising plasma levels of ANP to pharmacologic ranges might further decrease renal cGMP levels and tip the balance in favor of the inhibitory effect of ANP on compensatory growth. Indirect evidence that the response in plasma ANP levels and renal cGMP production to uninephrectomy is submaximal can be gleaned from the literature. Valentin and coworkers demonstrated that uninephrectomy prompts an acute doubling of the plasma ANP levels and urinary cGMP excretion in the rat (1). Wilkins and coworkers demonstrated that an infusion of lower doses of ANP than were used in this study caused a fivefold increase in plasma ANP levels and an eightfold increase in urinary cGMP (28). Therefore, it is possible that the pharmacologic effect on compensatory renal growth observed in this study might reflect augmentation of a physiologic role of ANP.

The types of cells labeled after in vivo injections of [3H]thymidine during compensatory renal growth have previously been evaluated (29). The majority of the mitoses involve proximal tubular cells with distal tubular cells, with collecting duct and loop of Henle cells comprising 17 and 10%, respectively. About 12% of the total number of mitoses reflect glomerular, capillary, and capsular cells. These studies did not include a specific evaluation of the effect of ANP on the distribution of mitoses. Such studies may contribute to the understanding of the mechanism of the antimitogenic effect of ANP. The receptor that
mediates the biologic effects of ANP is present in the glomerulus and every tubular segment but predominates in the inner medullary collecting duct (30). The glomerulus and inner medullary collecting duct are also the predominant sites of cGMP production in response to ANP (31). Therefore, specific suppression of mitoses in these sites by ANP would support the role of the cGMP-associated biologic receptor in the antimitotic effect of ANP.

The potential role of the clearance receptor for ANP in growth regulation has also been considered and recently summarized (21). Work in nonrenal tissue suggests that the clearance receptor may mediate the effect of ANP on growth. An evaluation of the effect of an infusion of C-ANP, an analogue of ANP with higher affinity for the clearance receptor than the biologic receptor, on compensatory renal growth would also be helpful in determining which receptor may be involved in growth suppression by ANP.

This study did not probe which growth factor or factors were influenced by ANP, but others have shown that ANP inhibits growth stimulated by platelet-derived growth factor and angiotensin II (3, 7). Angiotensin II exerts a mitogenic effect in mesangial cells (7), whereas it promotes hypertrophy of renal tubular cells (32). ANP has been shown to specifically oppose the mitogenic effect of angiotensin II on murine mesangial cells (7). ANP has also been shown to prevent angiotensin II-induced hypertrophy of vascular smooth muscle cells (33). This study revealed that ANP limited both mitogenic and hypertrophic responses to uninephrectomy, which is consistent with interference with the proposed growth effects of angiotensin II on both mesangial and renal tubular cells.

In summary, these experiments provide in vivo evidence of suppression of compensatory renal growth by ANP. Although the mechanism of the effect was not elucidated, the observation provides an in vivo correlation with recent in vitro observations from other laboratories, which together suggest that ANP may serve as a modulator of the effects of angiotensin II or other growth factors in compensatory renal growth.

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REFERENCES