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Effects of Atrial Natriuretic Factor on Hormone-Induced Renin Release

GILBERT DERAY, ROBERT A. BRANCH, WILLIAM A. HERZER, AKIHIRO OHNISHI, AND EDWIN K. JACKSON

SUMMARY The purpose of this study was to determine whether or not atrial natriuretic factor can act directly on the juxtaglomerular cell in vivo to inhibit hormone-induced renin release. To achieve this objective the interaction between synthetic atrial natriuretic factor and two different renin secretagogues was examined. To exclude any indirect effect of atrial natriuretic factor on renin release due to changes in sodium delivery to the macula densa, all studies were conducted in nonfiltering, canine kidneys. In one series of studies renin release was stimulated by intrarenal infusions of norepinephrine (3 μg/kg/min), and in a second series of studies renin release was induced by intrarenal infusions of prostacyclin (0.1 μg/kg/min). In both studies intrarenal infusions of atrial natriuretic factor (0.3 μg/kg/min), which provided supraphysiological levels of atrial natriuretic factor in the renal arterial plasma, failed to attenuate hormone-induced renin release. In contrast, adenosine, a well-known inhibitor of renin release, abolished the renin release response to both hormones. These data indicate that, at the dose used in this study, synthetic atrial natriuretic factor does not act directly on the juxtaglomerular cell to attenuate hormone-induced renin release. Further, these results imply that circulating endogenous atrial natriuretic factor cannot directly attenuate juxtaglomerular cell responsiveness. (Hypertension 9: 513-517, 1987)

KEY WORDS atrial natriuretic factor renin release prostacyclin norepinephrine adenosine

The atria of mammalian hearts contain a family of biologically active polypeptides called atrial natriuretic factor (ANF). ANF is stored in atrial-specific granules and is released into the bloodstream by atrial distention due to volume expansion. Circulating ANF may exert a number of physiologically important actions. In many respects ANF is the antithesis of the renin-angiotensin system. ANF is released by volume expansion, whereas renin is released by volume depletion. ANF causes diuresis and natriuresis; in contrast, low doses of angiotensin II decrease the ability of the kidney to excrete water and salt. ANF relaxes vascular smooth muscle, while angiotensin II contracts vascular smooth muscle. Further, ANF can attenuate several of the biological effects of angiotensin II, such as its vascular and steroidogenic actions.

The antithetical relationship between ANF and the renin-angiotensin system suggests that ANF may also influence the rate-limiting step in the formation of angiotensin II (i.e., the release of renin). Indeed, in general, intravenous infusions of ANF into animals with filtering kidneys decreases plasma renin activity (PRA). However, whether or not the suppression of renin release by ANF in vivo is due to a direct effect of the hormone on juxtaglomerular cells is unclear. In the filtering kidney ANF probably increases sodium chloride delivery to the macula densa, and this could account for the suppression of renin secretion by ANF in vivo. Unfortunately, in vitro studies have provided mixed results, with both ANF-induced inhibition and stimulation of renin release being reported. The explicit purpose of our study was to determine whether or not ANF exerts a direct effect in vivo to attenuate the responsiveness of juxtaglomerular cells.
to hormonal stimulation. The approach employed was to evaluate the effects of intrarenal infusions of synthetic ANF on the renin secretion response to direct activation of juxtaglomerular cells with either norepinephrine (NE) or prostacyclin (prostaglandin I₁, PGI₁). All studies were conducted in nonfiltering kidneys so that the natriuretic effects of ANF would not alter sodium chloride delivery to the macula densa and confound the interpretation of any observed inhibitory action of ANF. In addition, the effects of adenosine on hormone-induced renin release were determined to ensure that the renin release response in our model was suppressible.

Materials and Methods

Surgical Procedure

We studied a total of 20 mongrel dogs of either sex, weighing 11 to 26 kg. Each dog was anesthetized with pentobarbital (30 mg/kg i.v., supplemented as needed to maintain surgical anesthesia), and the left kidney was rendered nonfiltering as described by Blaine et al.16 With aseptic technique, the left renal artery, renal vein, and ureter were exposed through a flank incision. The ureter was ligated and severed, and the renal artery was occluded completely with a clamp that was removed after 2 hours. The incision was closed, and the dogs were allowed to recover. Animals were cared for according to institutional guidelines. Two or 3 days later the dogs again were anesthetized with pentobarbital (30 mg/kg i.v.). Food had been withheld for 24 hours before the experiment, but water was allowed ad libitum. After tracheal intubation the dogs were cared for according to institutional guidelines.

Study 1: Effect of ANF on Norepinephrine-Induced Renin Release

In Study 1, renin release was stimulated with intrarenal infusions of NE (3 μg/kg/min) in dogs receiving an intrarenal infusion of phenolamine (5 μg/kg/min beginning 1 hour before starting the protocol and continuing through the experiment). This infusion permitted direct activation of renin release through juxtaglomerular β-adrenergic receptors without a concomitant renin response secondary to α-adrenergic receptor-induced renal vasoconstriction. This approach also circumvented deterioration of renal hemodynamics caused by severe renal vasoconstriction. We previously have shown that the dose of phenolamine used was adequate to block α-adrenergic receptor–induced renal vasoconstriction.17

We used the approach of infusing NE in phenolamine-treated dogs instead of using the β-adrenergic-receptor agonist isoproterenol for two reasons. First, NE is an endogenous hormone mediating β-adrenergic receptor–induced renin release. Second, isoproterenol apparently stimulates renin release partially by activating extrarenal β-adrenergic receptors.18

The study design consisted of two 8-minute observation periods separated by 30 minutes. Mean arterial pressure (MAP) and RBF were monitored continuously, and 3-ml blood samples were obtained from the femoral artery and renal vein before and at the end of each 8-minute period. During each 8-minute period NE was infused into the renal artery through one of the 22-gauge needles. At all other times the NE solution was replaced with saline. Either saline or synthetic ANF was infused into the renal artery through the second 22-gauge needle at 0.3 μg/kg/min starting 10 minutes before and continuing throughout the 8-minute period. In some dogs, the renin response to NE in the presence of ANF was determined during the first infusion of NE and the control renin response to NE was determined during the second infusion of NE. In other dogs, this order was reversed. The order in any particular dog was chosen at random.

Study 2: Effect of Adenosine on Norepinephrine-Induced Renin Release

The protocol for Study 2 was the same as that described for Study 1 except that adenosine (30 μg/min) replaced the ANF infusion.

Study 3: Effect of ANF on PGI₁-Induced Renin Release

Study 3 protocol was the same as that described for Study 1 except that an intrarenal infusion of PGI₁ (0.1 μg/kg/min) replaced the NE infusion. In addition, dogs in this study did not receive an infusion of phenolamine. Instead, these animals were treated with propranolol (0.3 mg/kg as an i.v. bolus after the operation followed by a constant rate infusion of 5 μg/kg/min throughout the experiment). Because PGI₁ is cleared poorly by the lungs, even an intrarenal infusion can reduce blood pressure and reflexly increase the local and systemic release of catecholamines. The purpose of the propranolol treatment was to minimize β-adrenergic receptor–induced renin release so that the direct effects of PGI₁ on the juxtaglomerular cell could be studied. We previously have shown that the dose of propranolol used in this study blocks β-adrenergic receptor–stimulated renin release.17
Study 4: Effects of Adenosine on PGI$_2$-Induced Renin Release

The protocol for Study 4 was the same as that described for Study 3 except that adenosine (30 /ng/min) replaced the ANF infusion.

Quantitation of Renin Secretion

Blood samples for PRA determinations were collected in EDTA (0.3 ml of 10% EDTA per 10 ml of blood). Withdrawn blood was replaced by an equivalent volume of normal saline. Samples were placed immediately on ice and subsequently centrifuged at 4°C. The plasma was separated and stored at −20°C until assayed. PRA was determined by an angiotensin I radioimmunoassay as previously described, and the results were expressed as nanograms of angiotensin I generated at 37°C per milliliter of plasma per hour. Renin secretion rate was calculated by multiplying the arteriovenous difference of PRA by the renal plasma flow and was expressed as nanograms of angiotensin I per minute per hour.

Statistical Analysis

The results were analyzed using a two-factor analysis of variance in which one fixed factor was dose of ANF or adenosine and the second fixed factor was dose of NE or PGI$_2$. This analysis provided $p$ values for the overall main effects of ANF, adenosine, NE, and PGI$_2$, as well as for the interaction between ANF and NE or PGI$_2$; and the interaction between adenosine and NE or PGI$_2$. If a significant interaction was detected, the interaction was explored further by using paired Student’s $t$ tests. Statistical analyses were performed on an IBM PC AT (Armonk, NY, USA) using the number Crunchers Statistical System (Kaysville, UT, USA). All null hypotheses were two-tailed, and the criterion of significance was a level less than 0.05.

Drugs

Synthetic ANF (Arg 101–Tyr 126) was kindly provided by Dr. Edward Blaine (Merck Sharp & Dohme, West Point, PA, USA). The synthetic ANF was bioassayed on rat blood pressure before use, and activity comparable to other published results was observed. PGI$_2$ and NE were obtained from Sigma Chemical (St. Louis, MO, USA).

Results

Table 1 lists the values for RBF and MAP in all four studies. As indicated, neither ANF (Studies 1 and 3) nor adenosine (Studies 2 and 4) significantly altered RBF or MAP, although ANF tended to increase RBF. NE (Studies 1 and 2) did not influence RBF or MAP; however, PGI$_2$ (Studies 3 and 4) significantly increased RBF and significantly decreased MAP. Neither ANF nor adenosine significantly altered the effects of NE or PGI$_2$ on RBF or MAP (i.e., no statistically significant interactions were detected).

<table>
<thead>
<tr>
<th>Variable</th>
<th>RBF (ml/min)</th>
<th>MAP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>68 ± 7</td>
<td>87 ± 5</td>
</tr>
<tr>
<td>NE</td>
<td>78 ± 7</td>
<td>88 ± 3</td>
</tr>
<tr>
<td>ANF</td>
<td>94 ± 15</td>
<td>87 ± 7</td>
</tr>
<tr>
<td>NE</td>
<td>84 ± 14</td>
<td>88 ± 7</td>
</tr>
<tr>
<td>Study 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>110 ± 14</td>
<td>120 ± 6</td>
</tr>
<tr>
<td>NE</td>
<td>124 ± 12</td>
<td>125 ± 8</td>
</tr>
<tr>
<td>Adenosine</td>
<td>107 ± 7</td>
<td>125 ± 6</td>
</tr>
<tr>
<td>NE</td>
<td>133 ± 12</td>
<td>125 ± 7</td>
</tr>
<tr>
<td>Study 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>52 ± 7</td>
<td>108 ± 11</td>
</tr>
<tr>
<td>PGI$_2$</td>
<td>89 ± 21*</td>
<td>84 ± 14†</td>
</tr>
<tr>
<td>ANF</td>
<td>77 ± 13</td>
<td>112 ± 10†</td>
</tr>
<tr>
<td>PGI$_2$</td>
<td>96 ± 21*</td>
<td>76 ± 13†</td>
</tr>
<tr>
<td>Study 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>48 ± 10</td>
<td>104 ± 8</td>
</tr>
<tr>
<td>PGI$_2$</td>
<td>96 ± 16‡</td>
<td>72 ± 11‡</td>
</tr>
<tr>
<td>Adenosine</td>
<td>49 ± 5</td>
<td>96 ± 12</td>
</tr>
<tr>
<td>PGI$_2$</td>
<td>69 ± 16‡</td>
<td>71 ± 16‡</td>
</tr>
</tbody>
</table>

Values are means ± SEM for five animals. Interactions between effects were not significant by analysis of variance. RBF = renal blood flow; NE = norepinephrine; Study 1 = effect of ANF on response to NE; Study 2 = effect of adenosine on response to NE; Study 3 = effect of ANF on response to PGI$_2$; Study 4 = effect of adenosine on response to PGI$_2$.

* $p<0.1$, † $p<0.03$, ‡ $p<0.02$, compared with control values.

Figure 1 illustrates the effects of ANF and adenosine on NE-induced renin release. In Study 1 (left panel of Figure 1), NE significantly increased renin secretion. However, ANF did not exert an overall effect on renin release and did not alter the renin release response to NE. In contrast, in Study 2 (right panel of Figure 1), adenosine caused a reduction in the renin release response to NE, as indicated by the highly significant interaction term in the analysis of variance. In fact, NE failed to significantly increase renin release during the adenosine infusion, whereas it caused a marked increase during the saline infusion. Furthermore, baseline renin secretion was not significantly different in the presence and absence of adenosine, whereas NE-stimulated renin release was significantly lower during the infusion of adenosine.
FIGURE 1. Effects of synthetic ANF or adenosine on norepinephrine (NE)-induced renin release in the nonfiltering, \( \alpha \)-adrenergic receptor-blocked, canine kidney. The superscript \( \alpha \) indicates comparison between control period and nor-epinephrine infusion period; the superscript \( \beta \) indicates comparison between saline period and adenosine period (paired Student's \( t \) test). ANOVA = analysis of variance; AI = angiotensin 1; NS = nonsignificant.

FIGURE 2. Effects of synthetic ANF or adenosine on prostacyclin (PGI2)-induced renin release in the nonfiltering, \( \beta \)-adrenergic receptor-blocked, canine kidney. The superscript \( \alpha \) indicates comparison between control period and PGI2 period; the superscript \( \beta \) indicates comparison between saline period and adenosine period (paired Student's \( t \) test). See Figure 1 for key to abbreviations.

Figure 2 illustrates the effects of ANF and adenosine on PGI2-induced renin release. In Study 3 (left panel of Figure 2), PGI2 significantly increased renin release. In addition, ANF tended to increase the renin release response to PGI2, as suggested by the interaction term \( p<0.06 \) in the analysis of variance. This conclusion was strengthened by the observation that ANF did not significantly alter basal renin release, even though renin release during the PGI2 infusion seemed \( p<0.059 \) to be increased by the ANF infusion. These results indicate that ANF did not inhibit PGI2-induced renin release. Further, even though the data did not achieve the a priori criterion of a \( p \) level below 0.05, these results indicate a highly suggestive trend toward an ANF-induced increase in the renin response to PGI2. As shown in the right panel of Figure 2, all statements about the effects of adenosine on NE-induced renin release also apply to PGI2-induced renin release.

Discussion
The purpose of this study was to determine whether or not ANF can act directly on the juxtaglomerular cell in vivo to inhibit hormone-induced renin release. To exclude the role of the macula densa in renin secretion, the nonfiltering kidney model described by Blaine et al.16 was used. In this model intrarenal infusion of synthetic ANF failed to alter baseline renin secretion or to inhibit renin release induced by activation of juxtaglomerular PGI2 receptors or \( \beta \)-adrenergic receptors. These data are inconsistent with a direct action of synthetic ANF on the juxtaglomerular cell. If synthetic ANF and naturally occurring forms of the peptide are assumed to behave similarly, our data do not support a direct role for endogenous ANF in the control of renin release.

Three other explanations could account for the lack of effect of synthetic ANF on renin release in our study. First, renin release by our experimental preparation may not be responsive to inhibition by any hormone. However, this possibility can be excluded on the basis that adenosine abolished renin release under these conditions. Second, the lot of synthetic ANF we used might have chemically degraded and, therefore, become biologically inactive. To test this possibility, we examined the effect of our lot of synthetic ANF on blood pressure in the anesthetized rat. At an infusion rate of 1 \( \mu \)g/min, the lot of synthetic

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ANF used in this study decreased MAP in the rat by approximately 40% \((n = 5\) experiments), indicating full biological activity. A third explanation for the lack of effect of synthetic ANF on renin release in our study was that the dose used was insufficient. However, even if higher doses of synthetic ANF decrease renin release, this is unlikely to be of any physiological importance, since circulating levels of ANF are in the picogram per milliliter range, and the infusion rate used in our study provided nanogram per milliliter levels in the blood entering the kidney.

While this manuscript was in preparation, Opgenorth et al. \(^{22}\) reported that ANF, at the same dose used in our study, did not alter basal renin release in the nonfiltering canine kidney. Our results are in full agreement with the observation of Opgenorth et al. \(^{22}\) In addition, our results extend this earlier observation by demonstrating that ANF also does not alter hormone-induced renin release in the nonfiltering kidney.

In summary, our data demonstrate that synthetic ANF does not inhibit either PG\(_I\) or \(\beta\)-adrenergic receptor-induced renin release in the nonfiltering canine kidney. These results strongly suggest that endogenously released ANF does not directly influence juxtaglomerular cell function.

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**References**


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