Atrial Natriuretic Peptide Has No Potential to Protect Against Endotoxin-Induced Acute Renal Failure in the Absence of Renal Nerves

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Abstract. Atrial natriuretic peptide (ANP) has been shown to have the potential to restore renal function after ischemic injury, an underlying component of endotoxin (Et)-induced acute renal failure, and is known to counteract renal sympathetic nerve activity in renal function. We have recently found that renal denervation restores the Et-induced renal dysfunction. The purpose of this study was to examine effects of ANP infusion on the Et-induced acute renal failure in the absence of renal nerves. Ten to 14 days after bilateral renal denervation (DNX), Wistar rats (250 to 300 g body wt) were used in the acute experiment. Rats with intact renal nerves (INN) served as controls. Following control clearance measurements, rats were intravenously injected with 4 mg/kg Et (Escherichia coli, 055: B5). During endotoxemia, rats were infused with 10 µg/kg/h ANP or saline vehicle. Et injection reduced the glomerular filtration rate (GFR) significantly in saline-infused INN and DNX rats. ANP infusion restored the greatly reduced GFR to the pre-endotoxemia level in DNX rats but not in INN rats. There was significant difference between the ANP- and saline-infused DNX rats in the percentage change relative to the basal GFR value during the ANP infusion period. ANP infusion did not improve the hyponatriuresis and oliguria after Et administration, which is independent of renal nerves. In conclusion, ANP infusion has a minor reno-protective effect in rats with Et-induced acute renal failure in the absence of the renal nerves.

Key words: Acute renal failure, Atrial natriuretic peptide, Endotoxemia, Hypocapnia, Renal denervation, Sodium excretion

GRAM-negative bacterial infection commonly causes endotoxin (Et)-induced acute renal failure [1, 2]. Endotoxemia elicits cellular hypoxia [3, 4] and it has been suggested that it potentiates ischemic renal damage [5]. Most often, where Et is associated with acute renal failure, it appears to be largely acute tubular necrosis [6, 7]. Recent studies have reported beneficial effects of atrial natriuretic peptide (ANP) in limiting ischemic renal injury [8, 9]. In animal models of acute ischemic renal damage, ANP decreases renal vascular resistance [8, 10], increases renal blood flow [11, 12] and consequently, increases GFR [13, 14], which suggests a possible hemodynamic action of ANP in recovery from renal injury induced by an Et.

An increase in ANP release [15, 16] and higher sympathetic nerve activity [17, 18] are commonly observed neuroendocrine changes during endotoxemia that affect renal function. The interaction between these two responses on renal function has been described: An increase in efferent sympathetic activity produces decreases in GFR and renal blood flow [19, 20], and promotes renal vasoconstriction, antinatriuresis, and renin release.
[21, 22], all of which would counteract the effect of ANP on renal function. On the other hand, ANP significantly ameliorates norepinephrine-induced acute renal failure [23]. Recently we have found that renal denervation (DNX) restored the reduced GFR in endotoxemic rats to the level of saline-treated rats [24]. We therefore postulated that if ANP has the potential to improve renal dysfunction caused by Et, DNX would be helpful in displaying the renal effect of ANP. The purpose of this study was to confirm whether renal dysfunction and antinatriuresis caused by Escherichia coli Et administration are restored by ANP infusion in the absence of renal nerves.

**Methods**

The animals used in the present study were treated in accordance with the guidelines laid down for the care of laboratory animals by the authors' institution. Male Wistar rats underwent bilateral DNX (DNX rats) 10 to 14 days prior to the acute experiment. The kidneys were denervated by cutting all visible nerves entering the renal hilus and stripping the renal artery and vein of adventitia. The renal artery and vein were painted with a 10% solution of phenol in ethanol [25]. Control rats with innervated kidneys (INN rats) underwent sham operations. After these surgical procedures, all rats were fed the normal rat chow. Following a 10- to 14-day recovery period, each rat, weighing 250 to 300 g, was anesthetized by intraperitoneal injection of 50 mg/kg body wt sodium pentobarbital (Nembutal®, Abbott Laboratories, North Chicago, IL) and placed on a heated table for the duration of the experiment. A Silascon® catheter (ID 0.5 mm, OD 1.0 mm, Kaneka Medex, Kanagawa, Japan) was inserted into the superior vena cava for infusions and a polyethylene catheter (ID 0.5 mm, OD 1.0 mm, Natsume Seisakusho, Tokyo, Japan) was inserted into the carotid artery for blood sampling and blood pressure monitoring. The urinary bladder was cannulated with a polyethylene catheter (ID 1.2 mm, OD 1.7 mm) for urine collection. Tracheostomy was done and a polyethylene catheter (ID 1.5 mm, OD 2.7 mm) was placed in the trachea. A solution of 3% inulin in saline was infused at a rate of 1.5 ml/h throughout the experiment. Infusion of 6.25% albumin (bovine albumin, Sigma Chemical, St. Louis, MO) in lactate Ringer solution was started at a rate of 3% body wt/h for 30 min after the surgical procedures and was then decreased to 2% body wt/h. Sixty min after the acute surgical procedures, a 30-min control clearance period was commenced. Blood samples were withdrawn for serum inulin and electrolyte measurements at the end of the clearance period. After taking the serum samples, the packed cells were resuspended in saline and infused back into the rats. Following the control clearance period 4 mg/kg Et (E. coli 055: B5, Difco Laboratories, Detroit, MI) was injected into the rats intravenously. The rats were divided into two major groups, the ANP-infused group (n=14) and the time-control group (n=14).

![Experimental design. Et, Escherichia coli endotoxin; ANP, atrial natriuretic peptide; DNX, renal denervation; Surg, catheterization into the carotid artery, jugular vein, trachea and urinary bladder; Cl test, 30-min renal clearance test; Alb, albumin; BW, body weight.](image-url)
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Group (n=14). These groups were subdivided into the following four groups depending on whether the animals were INN or DNX (Fig. 1).

Group 1: DNX + Et + ANP (n=7). The rats were allowed a 150-min equilibration period after an injection of Et, after which 10 µg/kg/h synthetic human ANP (human atrial natriuretic peptide, Sigma Chemical) was infused. Ten min after the initiation of ANP infusion, a 30-min experimental clearance test was started.

Group 2: INN + Et + ANP (n=7). The protocol was the same as that for group 1, except that the renal nerves were intact.

Groups 3 and 4: Time controls for groups 1 (n=7) and 2 (n=7), respectively. All surgical procedures, clearance studies, measurements, and infusion rates for these groups of rats were exactly the same as for groups 1 and 2, except that saline was infused instead of ANP.

Analytical techniques

Plasma and serum were separated by centrifugation at 4 °C, and aliquots for each assay were stored at -70 °C until the study was completed. Arterial blood gas analysis was performed on an ABL-2 (Radiometer Corp., Copenhagen, Denmark). The GFR was calculated by using the inulin clearance data. Serum and urinary inulin concentrations were measured by the anthrone method [26]. Serum and urinary sodium concentrations were determined by an electrolysis method in an automatic analyzer (HITACHI 736: Hitachi Co. Ltd, Tokyo, Japan).

All values are presented as mean ± SEM. Comparisons within a group were made by ANOVA. Comparisons between ANP-infused and the time-control groups and between INN and DNX rats were made by unpaired t tests. P<0.05 was accepted as a statistically significant difference.

Results

Table 1 summarizes renal functions after Et administration in INN and DNX rats. Mean arterial pressure (MAP) was low after Et administration in all rats, except for the ANP-infused DNX rats. The GFRs in all INN and DNX rats were significantly reduced from the basal rates after Et administration.

Table 1. Renal function in endotoxemic rats after infusion of atrial natriuretic peptide in the presence and absence of renal nerves

<table>
<thead>
<tr>
<th></th>
<th>ET + ANP</th>
<th>Time-control</th>
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<tbody>
<tr>
<td></td>
<td>C</td>
<td>ANP</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>INN (7)</td>
<td>115 ± 5</td>
<td>91 ± 4*†</td>
</tr>
<tr>
<td>DNX (7)</td>
<td>111 ± 2</td>
<td>96 ± 10</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td></td>
<td></td>
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<tr>
<td>INN (7)</td>
<td>2.25 ± 0.41</td>
<td>0.94 ± 0.28†</td>
</tr>
<tr>
<td>DNX (7)</td>
<td>2.13 ± 0.29</td>
<td>1.98 ± 0.17†</td>
</tr>
<tr>
<td>V (µl/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>INN (7)</td>
<td>145 ± 24</td>
<td>38 ± 13†</td>
</tr>
<tr>
<td>DNX (7)</td>
<td>137 ± 18</td>
<td>63 ± 19</td>
</tr>
<tr>
<td>UNaV (µl/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>INN (7)</td>
<td>22.6 ± 5.5</td>
<td>5.1 ± 2.0†</td>
</tr>
<tr>
<td>DNX (7)</td>
<td>15.2 ± 1.8</td>
<td>7.0 ± 2.2†</td>
</tr>
<tr>
<td>FENa (%)</td>
<td></td>
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<tr>
<td>INN (7)</td>
<td>6.74 ± 0.78</td>
<td>3.43 ± 0.31†</td>
</tr>
<tr>
<td>DNX (7)</td>
<td>4.75 ± 0.93</td>
<td>2.65 ± 0.58</td>
</tr>
<tr>
<td>Reab/FilNa (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>INN (7)</td>
<td>93.3 ± 0.8</td>
<td>96.4 ± 0.3†</td>
</tr>
<tr>
<td>DNX (7)</td>
<td>94.1 ± 1.3</td>
<td>97.7 ± 0.6</td>
</tr>
</tbody>
</table>

All values are expressed as the means ± SEM. Numbers in parentheses are the numbers of animals used. Et + ANP, endotoxemic rats infused with atrial natriuretic peptide; C, value during control period; ANP, value during infusion of atrial natriuretic peptide; MAP, mean arterial pressure; GFR, glomerular filtration rate; V, urine flow rate; UNaV, urinary excretion of sodium; FENa, fractional excretion of sodium; Reab/FilNa, ratio of reabsorption to filtered load of sodium; INN, rats with innervated kidneys; DNX, rats with denervated kidneys. *significant difference from value for the corresponding period in the time-control group, P<0.05, unpaired t test; †significant difference from basal value within the group, P<0.05, ANOVA; ‡significant difference from value in innervated rats, P<0.05, unpaired t test.
INN rats exhibited decreased GFR in the experimental clearance period, which was not affected by ANP infusion: no return of GFR to the pre-Et control value was seen after ANP infusion. In contrast, in DNX rats in the time-control group, the reduced GFR after Et administration showed a tendency for restoration to the control value during the second clearance period. ANP infusion restored the GFR to the same level as that in the control period, and the value was significantly different from that in the time-control rats in comparison with the percentage change in the basal value (Fig. 2). The urine flow rate and urinary sodium excretion were noticeably reduced after injection of Et and were independent of the renal nerves in the ANP-infused and time-control rats. ANP infusion did not improve the hyponatriuresis and oliguria in INN and DNX rats. Although Et administration caused hyponatriuresis in all rats, the sodium reabsorption rate per filtered load remained unchanged and was not affected by ANP infusion (Table 1).

The results of arterial blood gas analyses are presented in Table 2. Et administration caused severe hypocapnia in experimental and time-control rats. In addition, Et treatment was associated with severe metabolic acidosis in INN and DNX rats: arterial bicarbonate ion concentrations and base excess were noticeably decreased after Et treatment compared with the basal values.

### Discussion

In the present study, a single intravenous injection of 4 mg/kg E. coli Et resulted in an extreme fall in the GFR in both INN and DNX rats, accompanied by systemic hypotension. The higher basal fractional sodium excretion in all rats was

![Fig. 2. Percentage changes relative to the basal glomerular filtration rate (GFR) value during infusion of atrial natriuretic peptide (ANP) at a rate of 10 µg/kg/h in the presence (INN) and absence (DNX) of renal nerves. Figures in parentheses are the numbers of animals used. *P<0.05 vs. basal GFR values, ANOVA.](image)

**Table 2.** Arterial blood gas values in endotoxemic rats after infusion of atrial natriuretic peptide in the presence and absence of renal nerves

<table>
<thead>
<tr>
<th></th>
<th>Et + ANP</th>
<th>Time-control</th>
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<tbody>
<tr>
<td></td>
<td>C (6)</td>
<td>ANP (8)</td>
</tr>
<tr>
<td>$P_aCO_2$ (mmHg)</td>
<td>37.4 ± 4.0</td>
<td>26.0 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>DNX (7)</td>
<td>44.2 ± 3.6</td>
</tr>
<tr>
<td>$HCO_3^-$ (mEq/l)</td>
<td>25.4 ± 1.3</td>
<td>16.6 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>DNX (7)</td>
<td>26.1 ± 1.2</td>
</tr>
<tr>
<td>$BE$ (mEq/l)</td>
<td>1.63 ± 0.65</td>
<td>-7.18 ± 1.14</td>
</tr>
<tr>
<td></td>
<td>DNX (7)</td>
<td>1.47 ± 0.91</td>
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</tbody>
</table>

All values are expressed as the means ± SEM. Numbers in parentheses are the numbers of animals used. Et + ANP, endotoxemic rats infused with atrial natriuretic peptide; C, value during control period; ANP, value during infusion of atrial natriuretic peptide; $P_aCO_2$, arterial $CO_2$ concentration; $HCO_3^-$, arterial bicarbonate ion concentration; $BE$, arterial base excess; INN, rats with innervated kidneys; DNX, rats with denervated kidneys. *significant difference from value for the corresponding period in the time-control group, $P<0.05$, unpaired $t$ test; †significant difference from control value within the group, $P<0.05$, ANOVA; ‡significant difference from value in innervated rats, $P<0.05$, unpaired $t$ test.
attributable to the volume expansion prior to Et administration in order to maintain blood pressure with infusion of a large volume of lactate Ringer solution. In this study, ANP infusion restored the reduced GFR after Et administration to the basal level in the absence of renal nerves, and the value expressed as a percentage change relative to the basal GFR value was significantly higher than that in the time-control rats. In contrast, GFR did not return to the pre-Et basal value after ANP infusion in the presence of renal nerves. The reno-protective effect of ANP during endotoxemia could therefore be traced in the absence of renal nerves, although the improvement in the GFR value was relatively minor, considering the massive pharmacological dose of ANP employed.

Recent studies show a therapeutic potential of the ANP for preserving renal function after renal ischemia [8, 9]. ANP administration immediately after the ischemic insult significantly improves renal structure and function as assessed by GFR and ATP generation [9, 10], the effects of which are not seen with other vasodilators such as acetylcholine [27] and nitroprusside [28]. The renal effect of ANP in the renal ischemic injury may be due to an increase in intraglomerular pressure which resulted from a unique combination of preglomerular arteriolar dilatation and efferent constriction [29]. Cellular hypoxia is an essential underlying pathophysiology for endotoxemia [3, 4], and renal impairment caused by ischemia and endotoxemia share common hemodynamic and metabolic changes such as reduced renal blood flow [2, 11, 12], increased renal vascular resistance [2], decreased renal energy charge [30], and increased serum and tissue lactate levels [30]. In addition, endotoxemia potentiates ischemic renal damage, thereby escalating a relatively modest ischemic insult to severe tubular necrosis [31]. Nevertheless, the results obtained in this study suggest that the pathophysiology underlying Et-induced acute renal failure may be multifactorial and not be attributable solely to the ischemic component. Irreversible direct or indirect cellular damage due to the substances activated by bacterial products such as polymorphonuclear leukocytes [32], tumor necrosis factor [33], interleukin-1 [34], platelet activating factor [35] and eicosanoids [36] are likely to be important in establishing the Et-induced renal failure.

Natriuretic and diuretic effects of ANP were also not seen in endotoxemic rats in this study. The urine flow rate and urinary sodium excretion were not affected by ANP infusion in INN and DNX rats. Intense stimulation of renal nerves, observed in endotoxia [17, 18], has been reported to counteract ANP in sodium and water excretion [37, 38], but the present study showed the renal nerve-independent attenuation of the natriuretic and diuretic effects of ANP during endotoxemia. There may be possible mechanisms whereby ANP infusion did not elicit natriuretic and diuretic effects after Et administration: 1) Hypotension: Recent studies suggest that the GFR and hypotension are independent [39, 40], but natriuresis and diuresis depend on the blood pressure [41, 42]. Failure to maintain MAP during endotoxemia appears to be a factor limiting the natriuretic and diuretic effects of ANP [43]; 2) Hypocapnia: Another possible factor is hypocapnia during endotoxemia, presumably due to hypermetabolism or compensation for metabolic acidosis in endotoxemic conditions. Hypocapnia is an inhibiting factor, directly or indirectly, of the natriuretic and diuretic effects of ANP [44, 45], which acts independently of the renal nerves [46]. Hyperventilation to produce hypocapnia stimulates renal nerve activity [47, 48] and causes antinatriuresis and antidiuresis to ANP. In our previous study, renal DNX restored the antinatriuresis after ANP infusion in hyperventilated normocapnia, but not in hyperventilated hypocapnia [46]. Hypocapnia during endotoxemia may be responsible for the attenuated natriuretic and diuretic effects of ANP.

In conclusion, 1) ANP infusion sustains the detrimental effect of Et on renal function in rats with intact renal nerves and has a reno-protective effect in rats with DNX kidneys. Given the fact that the dose of ANP used in this study was excessively large, the changes in renal function in DNX rats are minor; 2) ANP infusion does not improve the hyponatriuresis and oliguria after Et administration, which is independent of renal nerves. These data suggest that ANP has no therapeutic effect on renal dysfunction in endotoxemic patients.

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References

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