Effects of Atrial Natriuretic Peptide on Renal Function and Renin Release in the Isolated Perfused Rat Kidney

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Summary

The effects of synthetic atrial natriuretic peptide (ANP) on the renal hemodynamics, glomerular filtration rate (GFR), fluid and electrolyte excretion and renin release were studied in the isolated perfused rat kidney (IPK). When $10^{-9}$ mol of ANP was administered in 75 ml of perfusate, the renal vascular resistance (RVR) was transiently decreased for 3 to 5 min, thereafter increased for 30 min and then tended to return to the control level. ANP increased the GFR (0.55 ± 0.08 to 0.71 ± 0.07 ml/min), urine flow (UV) (0.018 ± 0.002 to 0.194 ± 0.028 ml/min), absolute Na excretion (UNaV) (1.83 ± 0.03 to 17.93 ± 2.71 μEq/min) and absolute K excretion (UKV) (0.67 ± 0.13 to 2.33 ± 0.18 μEq/min). The addition of indomethacin or mefenamic acid to the perfusate before the administration of ANP exerted no influence on any of the effects of ANP. Renin release was inhibited by approximately 50% compared to the ANP-free control group. With the administration of ANP, UV and UNaV reached a peak 15–20 min after the GFR reached a peak and remained elevated after the GFR fell below the control level. These findings suggest that $10^{-9}$ mol of ANP causes natriuresis and renin suppression in the IPK, and that the natriuresis is prostaglandin-independent and cannot be explained only by an increase in GFR.

Additional Indexing Words:
Renal vascular resistance Glomerular filtration rate Diuresis Natriuresis Prostaglandins Medullary blood flow

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RECENT evidence has shown that synthetic atrial natriuretic peptides (ANP) can cause marked diuresis and natriuresis both in humans and in experimental animals. In spite of these intensive studies, the effects of ANP on the glomerular filtration rate (GFR) and renal blood flow (RBF) remain controversial. One possible factor in the disparate results is that the multiple effects of ANP on the renal hemodynamics are such that they can relax the vascular smooth muscle, inhibit the vasoconstrictive actions of angiotensin II and norepinephrine and reduce the secretion of renin and aldosterone.

Since it is not easy to determine the exact actions on renal hemodynamics, the present study employed the isolated perfused rat kidney (IPK), in which neural and systemic hemodynamic effects can be eliminated, in order to clarify the direct action of ANP on renal hemodynamics as well as on renin release.

METHODS

Kidney perfusion:

The perfusion apparatus and operative procedures were identical to those described by Nakane et al. Briefly, under intraperitoneal pentobarbital anesthesia (50 mg/Kg), the right ureter of a male Wistar rat weighing 250–350 g was catheterized with PE 10 tubing, and the right renal artery was cannulated with an 18 G needle via the superior mesenteric artery. The right kidney was then isolated and perfused with 75 ml of blood-free Krebs-Ringer bicarbonate buffer (pH 7.4), containing 5.5 mM glucose, 5% bovine serum albumin (Fraction V, Sigma Co., USA) and 1.0 mg/dl of creatinine, which was added for the measurement of GFR. The initial perfusion pressure (PP) was maintained at 90–100 mmHg.

The renal perfusate flow (RPF) was measured by a flowmeter inserted between the peristaltic pump and the renal artery in the perfusion circuit, and the PP was determined by previously described methods.

Experimental protocol:

After allowing a 20-min equilibration period, two 10-min control clearance periods were recorded. At the end of the control period, 10⁻⁹ mol of synthetic rat ANP which is known to be composed of 28 amino acids (Peninsula Lab., USA) was administered in the perfusate and five 10-min experimental clearance periods were initiated. This procedure was performed in 5 perfused kidneys. Further, in order to examine whether the effects of ANP depend on prostaglandins, at the end of each of the two 10-min control clearance periods, indomethacin (2.8 × 10⁻⁶ mol) or mefenamic
acid \((4.0 \times 10^{-6} \text{ mol})\) was added to the perfusate of 4 and 3 kidneys, respectively. Kidneys which were perfused without special reagents were used as the control group \((n=6)\). Perfusate was collected at the midpoint of each control and experimental clearance period, and urine was collected for every 10-min period.

**Measurements:**

The renal vascular resistance (RVR) was calculated from \(\text{PP}/\text{RPF}\) and expressed as \(\text{mmHg (ml/min)}^{-1}\). GFR was determined as the creatinine clearance which was estimated with an autoanalyser (Beckman ASTRA 4). Na and K were measured using iso-selective electrodes. The urine volume was determined gravimetrically. The perfusate renin concentration (PRC) was estimated by radioimmunoassay.\(^9\)

**Statistics:**

All results are expressed as mean \(\pm\) SE. For statistical analysis, standard methods were applied including co-variance analysis. P values of less than 0.05 were taken as significant.

**Results**

Figs. 1–4 illustrate the changes in RVR, GFR, urine flow (UV), UNaV, UKV, the fractional excretion of Na (FENa) and PRC resulting from the

![Graph](image)

**Fig. 1.** Time course of effects of ANP on renal vascular resistance (RVR) and on glomerular filtration rate (GFR) compared to control. (All following figures are compared with the control group.)
Fig. 2. The changes in urine volume (UV) and urinary excretions of sodium (UNaV) and potassium (UKV) induced by ANP are shown in the 3 panels.

Fig. 3. Time course alterations in fraction of sodium excretion calculated by UNaV/perfusate sodium concentration × GFR by administration of ANP are illustrated.
administration of 10⁻⁹ mol of synthetic ANP in 75 ml of perfusate in the IPK.

Initially, the RVR was decreased by 2% for 5 min, followed by a gradual increase, reaching a peak at 30 min after the administration of ANP. ANP significantly increased the GFR (0.545±0.079 to 0.713±0.072 ml/min), UV (0.018±0.002 to 0.194±0.028 ml/min), UNaV (1.83±0.03 to 17.93±2.71 μEq/min) and UKV (0.67±0.13 to 2.33±0.18 μEq/min). The addition of indomethacin or mefenamic acid to the perfusate containing ANP exerted no influence on any of the effects of ANP (data not shown). UV and UNaV reached peak levels at 12–20 min after the GFR peak and remained elevated beyond the time when the GFR fell below the control level.

Renin release was inhibited by approximately 50% compared to the control group.

**DISCUSSION**

This study clearly demonstrated that, in the isolated perfused kidney, synthetic atrial natriuretic peptide caused a transient decrease in renal vascular resistance followed by a marked increase. Further, in spite of initial decreases in RVR after the administration of ANP, the glomerular filtration rate became progressively elevated, accompanied by marked increases in urine volume and urinary sodium excretion. These results are in good accordance with previous in vivo and in vitro studies.⁴,¹⁰–¹² The mechanisms by which ANP induced prominent increases in GFR remain controversial. In the present study, we employed an IPK system, since several factors such as changes in systemic blood pressure, activation of the sympathetic nervous system and increases in angiotensin II can thus be eliminated. It seems probable therefore that the gradual increases in GFR accompanied by increased FENa were directly related to an increase in the RVR. Furthermore, since the changes
in RVR were biphasic, viz. a transient initial decrease followed by a marked elevation, it is thought that the ANP first produced a decrease in preglomerular resistance. However, a direct effect of ANP increasing the filtration coefficient (Kf) and resulting in an increased GFR cannot be excluded. Another important finding in this study was that UV and UNaV were continuously elevated after the GFR had fallen to the control level. One possible mechanism is a direct action of ANP on the renal tubules of the IPK by either inhibiting sodium reabsorption in the proximal tubules or inhibiting chloride reabsorption in Henle's loop, although several reports of in vitro studies have rejected a direct action of ANP on the renal tubules.\(^{13,14}\) Another possible mechanism is that ANP releases vasoactive substances such as prostaglandins which are known to modulate the renal handling of water and sodium balance. To our knowledge, there have been no conclusive data demonstrating active involvement of vasoactive substances in relation to the action of ANP.\(^{13}\) Furthermore, the present results show that prostaglandins do not play any appreciable role related to the action of ANP. A more attractive explanation for the discrepancy between GFR and sodium excretion would be an increase in medullary blood flow, since Borenstein et al\(^{16}\) clearly demonstrated this phenomenon using the microsphere and albumin uptake methods.

In addition to these renal hemodynamic studies in the IPK, measurements of the perfusate renin concentration after ANP administration were made. Recent investigations have shown that intravenously or intrarenally administered ANP inhibits renin release in spite of massive diuresis and natriuresis.\(^{21,23}\) In the IPK system, we confirmed these inhibitory effects of ANP on renin release. Combining these and previous data,\(^{17,19}\) since indirect inhibition by enhanced delivery of sodium chloride to the macula densa is improbable, it seems most likely that ANP exerts a direct inhibitory action on the juxtaglomerular cells. However, the exact mechanisms involved in the stimulation of renin release by ANP remain unclear and further studies are needed to clarify the details.

REFERENCES

4. Seymour AA, Blaine EH, Mazack EK, Smith SG, Stabilito II, Haley AB, Napier MA, Whin-
nery MA, Nutt RF: Renal and systemic effects of synthetic atrial natriuretic factor. Life Science 36: 33, 1985