Atrial Natriuretic Peptide Attenuates the Development of Hypertension in 2-Kidney, 1-Clip Goldblatt Rats*

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ABSTRACT

Effects of atrial natriuretic peptide (ANP) on the development of hypertension in 2-kidney, 1-clip (2-K, 1-C) rats were examined. In one group of rats, ANP infusion (500 ng/hr, iv) started immediately after clipping the renal artery. Another group of rats with one kidney-clipped was without ANP infusion and served as a control. Blood pressure was measured on days 4, 7, and 10 following clipping the renal artery. Upon the last blood pressure measurement finished, blood sample was collected by decapitation to measure plasma renin activity (PRA), and both kidneys were taken to weigh and to measure renin content. The ANP-infused group showed an attenuation of increases in blood pressure compared to the non-infused control group. PRA was lower in the ANP-infused group than in the non-infused group. Cortical renal renin content (RRC) of the clipped kidneys was not different between ANP-infused and non-infused groups. The clipped kidneys showed a higher RRC and weighed less than the non-clipped contralateral kidneys within each group. In contrast, sham-clipped rats did not show significant changes in any of the parameters examined regardless of whether ANP was infused or not. These results demonstrate that chronic ANP infusion does not prevent but does attenuate the development of hypertension in 2-K, 1-C rats. It is suggested that ANP plays a role in the long-term regulation of blood pressure, at least in part, by antagonizing the renin-angiotensin-system.

. **Key Words**: Atrial natriuretic peptide; 2-K, 1-C hypertension

INTRODUCTION

Atrial natriuretic peptide (ANP) is a potent vasoactive and natriuretic principle present in atrial specific granules (DeBold, 1982; Garcia et al, 1982). Garcia et al (1985a, 1986) examined the effect of chronic infusion of ANP in established two-kidney, one-clip (2-K, 1-C) hypertensive rats, and concluded that the hypotensive response to ANP is mainly due to direct vasodilation. However, one may not rule out a role of the associated decrease in plasma renin activity (PRA) in lowering the blood pressure, at least in part, in this model of hypertension.

Two-kidney, one-clip hypertension has been attributed to an increased activity of renin-angiotensin-system (RAS) (Singer et al, 1963; Van der Wal & DeJong, 1975; DeForrest et al, 1979). On the other hand, ANP has been reported to suppress the release of renin from the kidney (Maack et al, 1984; Lappe et al, 1985). Thus taking into account the relationship between RAS and what is presumed to be its target effect, blood pressure, the decrease in PRA may play a causal role to decrease the blood pressure in ANP-infused 2-K, 1-C rats.

Therefore, if RAS were to be prevented to develop

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its full capacity by ANP in 2-K, 1-C rats, development of hypertension may also be affected. The present study was aimed to test the hypothesis that ANP attenuates the development of hypertension in 2-K, 1-C rats.

METHODS

Two-kidney, one-clip hypertension was made in male Sprague-Dawley rats (210~250 g) by constriction of the left renal artery with a silver clip having an internal gap of 0.25 mm; the contralateral kidney was left untouched. The rats were, then, divided into two separate groups. The one group (Clip+ANP) was infused with ANP (atriopeptin III, Peninsula; 500 ng/hr) through a subcutaneously implanted osmotic pump (model 2ML2, Alza, Palo Alto, CA) in the neck and a polyethylene catheter (PE-60) connected to the left jugular vein. ANP infusion started immediately after clipping the artery. The other group (Clip-ANP) was implanted with a saline-filled pump connected to the jugular vein.

Two additional groups of rats, subjected to a sham operation, were also provided. The one (Sham+ANP) was infused with ANP as were the ANP-infused 2-K, 1-C rats, and the other (Sham-ANP) was without ANP-infusion.

The animals were kept on a regular rat chow and tap water *ad libitum*.

Systolic blood pressure (SBP) was measured indirectly by means of a tail cuff method in conscious, pre-warmed (37℃ for 10 minutes) rats. The basal blood pressure was obtained as an average of 3 or 4 consecutive-day-values before clipping the renal artery. After clipping the artery, SBP was measured on days 4, 7 and 10.

On day 10, after measuring SBP, the animals were decapitated and trunk blood was collected for measurement of PRA. Both kidneys were removed and weighed; cortical slices from each were then prepared and homogenized in an ice-cold aqueous solution of bovine serum albumin (0.1%) as described previously (Katz & Malvin, 1984).

PRA and renal renin contents (RRC) using prepared homogenates were measured by radioimmunoassay of generated angiotensin I.

Results were expressed as means±SE. Each datum point (SBP) was compared by unpaired Student *t*-test between ANP-infused and non-infused groups. Likewise, PRA and RRC were also compared between the groups.

RESULTS

Blood pressure measurements

Fig. 1 shows the development of hypertension in 2-K, 1-C rats. ANP-infused group (Clip+ANP) showed a lesser degree of increase in blood pressure than did the non-infused control group (Clip-ANP). No significant changes in blood pressure were observed in sham-operated rats, regardless of whether

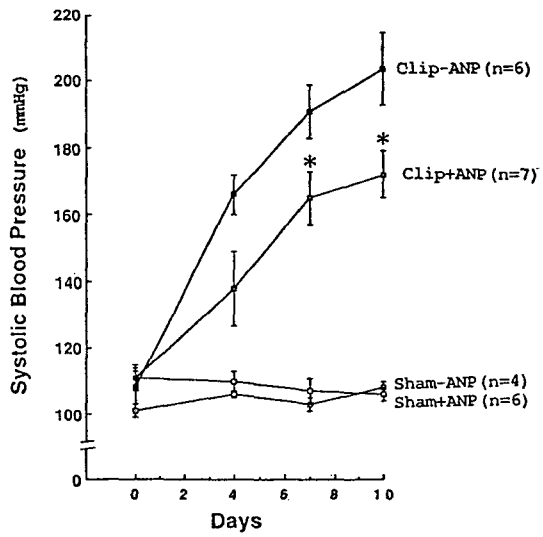

Fig. 1. Effect of atrial natriuretic peptide (ANP)on the development of hypertension in 2-kidney, 1-clip rats. Blood pressure was not modified by ANP infusion in sham-operated rats. *$P<0.05$, compared to the non-infused rats (Clip-ANP) on the corresponding day after clipping the renal artery.

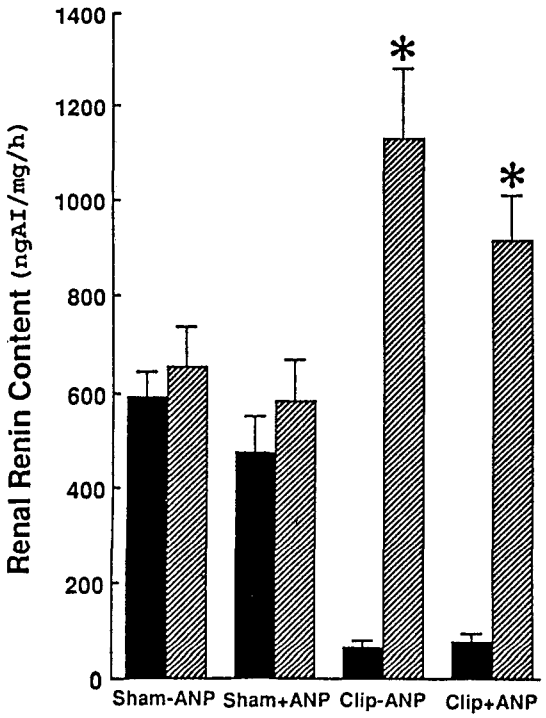

Fig. 2. Cortical renal renin content in four groups of rats. Hatched bars represent the clipped kidneys and black bars the contralateral non-clipped kidneys. *P<0.01, compared to the nonclipped opposite kidney. The values were not significantly different between the clipped kidneys.

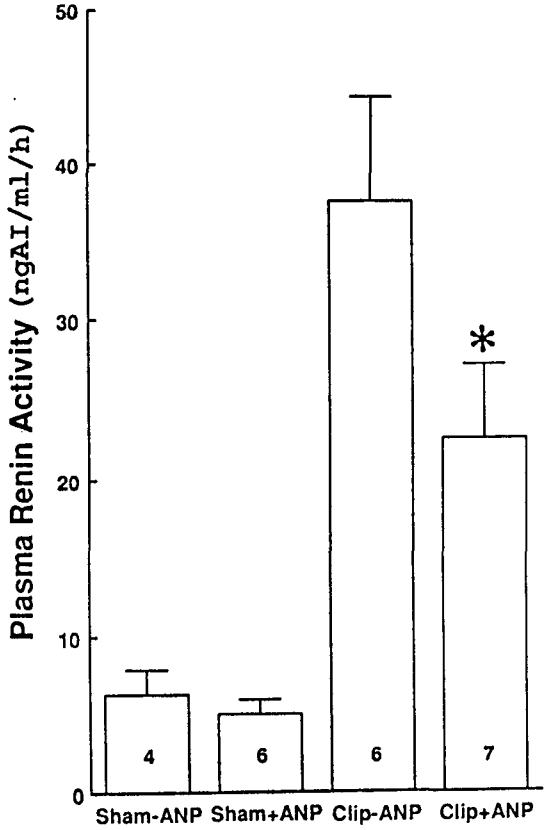

Fig. 3. Plasma renin activity in four groups of rats. *P< 0.05, compared to the non-infused rats (Clip-ANP).

ANP was infused or not.

Renal renin contents

Fig. 2 represents the cortical RRC. In ANP-infused as well as non-infused group, RRC of the clipped kidneys were significantly higher than those of the contralateral non-clipped ones. However, comparing the ANP-infused (Clip+ANP) to the non-infused (Clip-ANP) group, RRC were significantly different neither between the clipped nor between the non-clipped kidneys. In sham-operated rats, no difference in RRC was observed between the kidneys regardless of ANP infusion.

Plasma renin activity

PRA measured on the 10th day after clipping the renal artery was lower in the ANP-infused (Clip+ANP) than in the non-infused (Clip-ANP) rats(Fig. 3). However, both clipped groups showed a higher PRA than sham-operated rats, in which PRA was not affected by the ANP-infused: PRA in the ANP-infused (Sham+ANP) was comparable to that in the non-infused (Sham+ANP) group.

Kidney weights

Table 1 shows the kidney weights in 4 different groups of rats. Clipped kidneys weighed significantly less than did the contralateral non-clipped. In sham-operated rats, right and left kidneys did not weigh differently.

Table 1. Right and left kidney weights in four different groups of rats

Group	Right (g)	Left (g)
Sham−ANP (n=4)	1.15±0.05	1.16±0.04
Sham+ANP (n=6)	1.04±0.03	1.01±0.03
Clip−ANP (n=6)	1.36±0.05	0.92±0.10**
Clip+ANP (n=7)	1.36±0.08	1.03±0.06*

*p<0.05, **p<0.01: Compared to the right kidney. n=number of animals.

DISCUSSION

It has been shown that chronic infusion of ANP produces a gradual reduction of blood pressure to normal levels in established 2-K, 1-C hypertensive rats (Garcia et al, 1985a; 1986). The normalization of blood pressure was not accompanied by an increased natriuresis or diuresis but was associated with a lower PRA, at least in part.

Indeed evidence has been accumulated to suggest a functional relationship of ANP to RAS. The hypotensive response to ANP has been reported to be greater or observed only in saralasin-sensitive 2-K, 1-C rats (Volpe et al, 1985; Garcia et al, 1987). Furthermore, ANP antagonizes angiotensin II-induced contraction of isolated vascular preparations more profoundly than that produced by norepinephrine (Kleinert et al, 1984), and specifically inhibits intracerebroventricular angiotensin II-induced thirst or pressor response (Nakamura et al, 1985a).

The hypothesis tested in the present study was that ANP attenuates the development of hypertension in 2-K, 1-C rats by inhibiting RAS. PRA measured on the 10th day after clipping the renal artery was significantly lower and the development of hypertension attenuated in the ANP-infused compared to the non-infused 2-K, 1-C rats. It has been recognized that the early phase of 2-K, 1-C hypertension is associated with an enhanced activity of RAS (Morton & Wallace, 1983). On the other hand, ANP suppresses the release of renin from the kidney (Maack et al, 1984; Lappe et al, 1985). Taken together, the attenuated increase of blood pressure in the ANP-infused rats may be attributable to a suppression of RAS to develop its full capacity by the ANP-infused.

There may be several points of potential control of renin synthesis or secretion. One may therefore argue that the lower PRA in ANP-infused group is not primarily due to a suppressed release of renin, but is secondary to a direct inhibitory effect of ANP on renin synthesis. However, the present data may rule out such a speculation, since RRC of the clipped kidneys were comparable between ANP-infused and non-infused rats.

Nakamura et al (1985b) have shown that changes in plasma renin levels and RRC are non-proportional. They suggested that stimulation of the RAS occurs most likely at the transcriptional level with rapid release of newly synthesized renin and limited, if any, storage. However, in the same study, after inhibition of the highly stimulated RAS was there a decrease of plasma renin concentration without significant changes in either RRC or renin mRNA content. These results point to a post-translational mechanism of inhibition and exclude an effect on membrane transport rate or granular extrusion. The present study is also in keeping with the possibility that ANP inhibits renin secretion at the post-translational stage, since a decrease of PRA was noted in the ANP-infused rats with no significant decrease in RRC.

The lower PRA in ANP-infused 2-K, 1-C rats cannot be ascribed to any technical problem in clipping the renal artery, since the clipped kidneys weighed less than the contralateral non-clipped kidneys on the order of what did less in non-infused control rats.

Although many researchers are in agreement with that ANP decreases PRA and renin secretion rate,

Volpe et al (1985) reported an increase in PRA by the ANP-infused in 2-K, 1-C rats. They attributed this failure of ANP to decrease PRA to an impaired ability of the peptide to increase sodium chloride delivery promptly in the ischemic renin-secreting kidney. However, they observed a decrease in plasma aldosterone levels in the same experiment, which probably resulted from a direct adrenal effect of ANP (Atarashi et al, 1984; Chartier et al, 1984; DeLean et al, 1984). After all, their results could not completely rule out a possible role of ANP for the changes in the cascade of RAS to decrease the blood pressure. Whether or not there was also a decrease in aldosterone secretion due to a direct inhibitory effect on adrenal cells by ANP is not certain in the present study.

As others (Garcia et al, 1985b; 1986) have previously reported in chronically ANP-infused normotensive rats, ANP did not modify blood pressure in sham-operated animals. Therefore, it is unlikely that a decrease in cardiac output by ANP, as such has been demonstrated in acute experiments (Lappe et al, 1985), played a role in attenuating the development of hypertension in the present study. Nor is it likely that a peripheral vasodilation was responsible.

Accordingly, PRA was not modified by the ANP-infused in sham-operated animals:PRA in the ANP-infused group was comparable to that in the non-infused. This observation is not in agreement with those where acute administration of ANP reduced renin secretory rate (Burnett et al, 1984; Maack et al, 1984). However, their experiments were done in anesthetized animals, in which renin secretory rate may have already been stimulated. ANP may reduce PRA only when it is initially or being stimulated. The discrepancy may also be due to the difference in the manner of ANP infusion: an acute versus a chronic infusion.

In summary, the present study demonstrates that chronic ANP infusion does not totally prevent but attenuates the development of hypertension in 2-K, 1-C rats. It is suggested that ANP plays a role in the long term regulation of blood pressure, at least in part, by antagonizing the RAS. However, it is still a long way from these speculations to a clear understanding of the relationship of the potential role of ANP to the regulation of cardiovascular function.

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═ 국문초록 ═

2-Kidney, 1-Clip Goldblatt 흰쥐에 있어서 고혈압 발생에 미치는 Atrial Natriuretic Peptide 의 영향

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이 종 은

Atrial natriuretic peptide (ANP)의 혈압조절에 대한 효과를 구명하기 위하여 2-kidney, 1-clip 흰쥐의 고혈압 발생에 미치는 ANP의 영향을 조사하였다. 실험군은 왼쪽 신동맥에 clip을 끼우고 경정맥내에 지속적으로 ANP를 주입한(atriopeptin III, 500 ng/h) 군으로 하였으며, 대조군은 clip을 끼웠으나 ANP를 주입하지 않은 군으로 하였다. Clip을 끼우기 전과 clip을 끼운 제 4,7,10일에 꼬리동맥으로부터 혈압을 측정한 후 단두하여 혈액을 채취하고 양측 신장을 떼어내어 renin 치를 구하였다. ANP 주입군은 대조군에 비하여 고혈압의 발생이 약화되었고 혈장 renin 활성도가 유의하게 낮았으나 신피질 renin함량은 양군간에 유의한 차이가 없었다. 한편 clip을 끼우지 않은 흰쥐에서는 ANP 주입군과 비주입군간에 혈압, 혈장 renin 활성도와 신피질 renin 함량 등이 모두 서로 유의한 차이를 보이지 않았다. 이상의 실험결과는 지속적 ANP 주입이 2-K, 1-C 흰쥐의 고혈압 발생을 방지하지는 못하였으나 약화시켰음을 보여주었으며, ANP는 적어도 부분적으로는 renin-angiotensin계를 길항하므로써 장기적 혈압 조절에 관여하는 것이 시사되었다.