PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 178, 155-159 (1985)

# EFFECT OF CHRONIC INFUSION OF SYNTHETIC ATRIAL NATRIURETIC FACTOR (ANF 8-33) IN CONSCIOUS TWO-KIDNEY, ONE-CLIP HYPERTENSIVE RATS

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Abstract. Conscious two-kidney, one-clip hypertensive rats were chronically infused during 7 days with synthetic ANF (8-33) (1 μg/hr/rat) by means of osmotic minipumps. The initial blood pressure of 183 ± 4 mmHg gradually decreased to 116 ± 5 mmHg the last 2 days of infusion. Pressure diuresis returned to normal and pressure natriuresis was attenuated. PRA was significantly lower (1.81 ± 0.41 AI ng/ml/hr) than in not treated hypertensive rats (8.56 ± 3.75 AI ng/ml/hr). A partial regression in cardiac hypertrophy was observed in the treated group. We suggest that the hypotensive response to ANF may be mainly due to vasodilatation, but the possibility that the decrease in PRA may play a partial role in lowering blood pressure, cannot be excluded. 

Introduction. Atrial natriuretic factor (ANF) is a potent vasoactive and natriuretic peptide present in atrial specific granules (1-2) which has been recently purified to homogeneity and sequenced (3-9). A peptide with the amino acid sequence (8-33AA) of native ANF and with similar natriuretic and vasoactive activities has been synthesized (6,10,11).

We have recently demonstrated (13) that the acute administration of this synthetic ANF produced a rapid, fast and prolonged decrease in blood pressure in renovascular hypertensive rats which is probably not due to an increased natriuresis but to vasodilatation. We have now investigated the effect of chronic infusion of the synthetic peptide in the conscious two-kidney, one-clip (2-K,1-C) hypertensive rat.

Materials and Methods. Two-kidney, one-clip hypertension was produced in male Sprague-Dawley rats (180-200 g) by constriction of the left renal artery with a silver clip having an internal gap of 0.20 mm; the contralateral kidney was left untouched. One additional group of rats subjected to a sham operation in which the left kidney was exposed and the renal artery stripped, was used as normotensive control.

Blood pressure was measured indirectly twice a week by means of a tail cuff under light ether anesthesia, and recorded on a Grass model 7 polygraph fitted with a 7P8 preamplifier and a model 1010 grass crystal microphone as a pulse detector. Once the blood pressure of 2-K, 1-C rats was 150 mmHg or higher during 4 consecutive weeks, the animals were accomodated in individual metabolic cages and allowed 3 to 4 days to become accustomed to their new environment. The animals were kept on regular rat chow and tap water *ad libitum*. Twenty-four hours after this initial period the animals were separated in three experimental groups. Under light ether anesthesia, one group of 2-K, 1-C hypertensive rats was subcutaneously implanted in the neck with osmotic minipumps (model 2001, alza, Palo Alto, CA) filled with ANF 8-33 to release 1 μg/hr of the peptide. The pumps were connected to the left jugular vein by means of a polyethylene catheter (PE-60). Sham-operated rats and a second group of 2-K,



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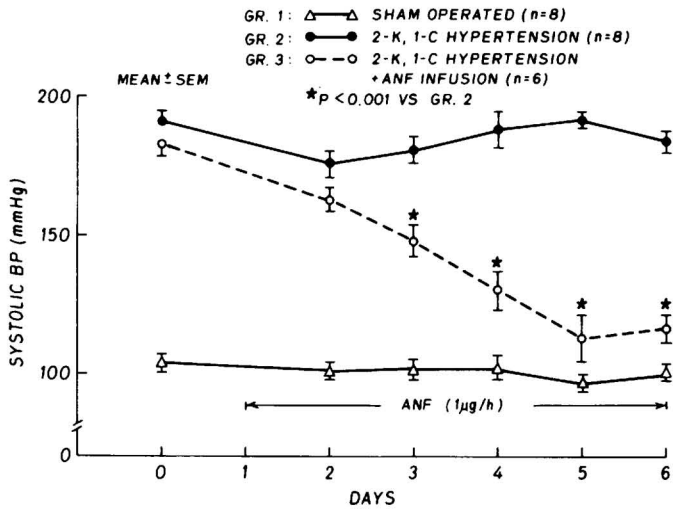


Figure 1 Effect of ANF infusion on blood pressure of the 2-K, 1-C hypertensive rats.

1-C hypertensive rats, were equally anesthetized and a piece of plastic tubing with the same size and diameter as the minipump was subcutaneously implanted. The left jugular vein was cannulated with a blind PE-60 catheter.

Urine volume, water intake, weight and indirect blood pressure were measured daily. Urinary sodium and potassium were measured daily in a flame photometer.

On day seven after the pumps were installed, the animals were decapitated and blood collected, the heart was excised ans weighed. Plasma renin activity was measured by radioimmunoassay of generated angiotensin I (12).

Results are expressed as means ± SEM. Single comparisons were done by the paired and unpaired Student's "t" test. One-way analysis of variance, analysis of covariance, and the Dunnet test were used for multiple comparisons.

Results. No difference in initial blood pressure was seen between 2-K, 1-C and 2-K, 1-C ANF-infused hypertensive groups: 191 ± 4 mmHg in the former and 183 ± 5 mmHg in the latter. As seen in Fig. 1, blood pressure in the ANF-infused group gradually declined, the decrease becoming significant 48 hr after the pumps were installed. Blood pressure reached levels not significantly different from those of the sham-operated group during the last two days of the experiment.

A significant decline in urinary volume and water intake (Fig. 2b and 2c) was observed in the 2-K, 1-C rats infused with ANF. Their initial levels before treatment were not significantly different from those of the 2-K, 1-C not infused group. A similar trend was observed for natriuresis (Fig. 2a) but because of intra-group variations the difference was not significant. No such decline was observed in either normotensive or hypertensive control groups.

As seen in Table I, 2-K, 1-C ANF-infused rats presented PRA levels significantly lower than those hypertensive animals which were not infused with ANF, and similar to those of sham-operated rats. In the same table it can be seen that in not infused 2-K, 1-C hypertensive rats the cardiac weight is significantly higher than in sham-operated rats. Heart weight in ANF-infused hypertensive rats is not significantly different from that of the control group.

Discussion. We have previously shown (13) that the acute administration of a $ED_{50}$ diuretic dose of synthetic ANF produced a decrease in blood pressure in normotensive and renovascular hypertensive rats which was not ascribed to a reduction in circulating volume but to vasodilatation. This effect was more pronounced in the 2-K, 1-C hypertensive group.

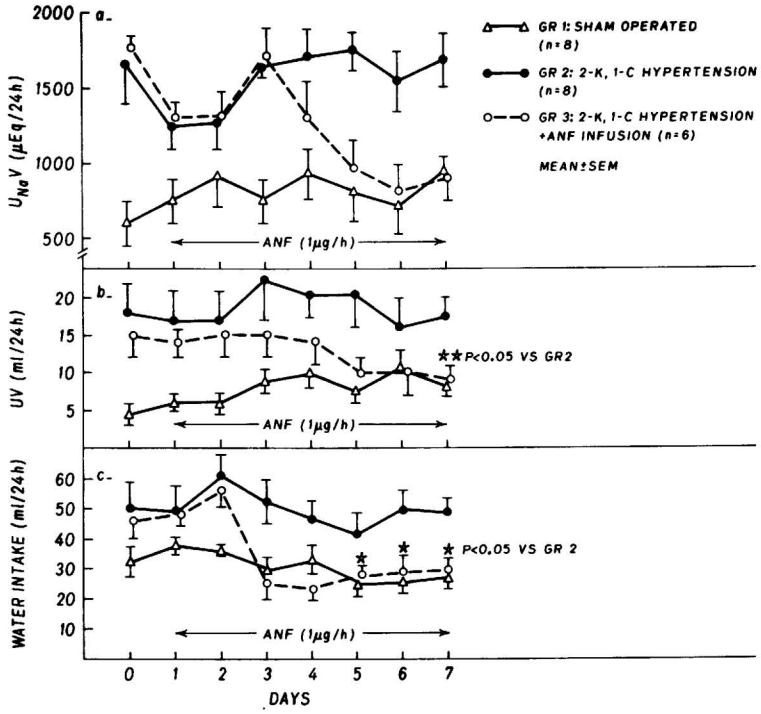


Figure 2 Effect of ANF infusion on natriuresis (a), diuresis (b) and water intake (c) in the 2-K, 1-C hypertensive rats.

Table I

EFFECT OF CHRONIC INFUSION OF SYNTHETIC ANF 8-33 ON PRA AND HEART WEIGHT IN THE 2-K, 1-C HYPERTENSIVE RAT

| Group | PRA (AI ng/ml/h) | Heart weight (g) |
|---|---|---|
| Sham-operated | 1.47 ± 0.29 | 0.94 ± 0.12 |
| | n = 8 | n = 8 |
| 2-K, 1-C | 8.56 ± 3.75* | 1.30 ± 0.07** |
| | n = 8 | n = 7 |
| 2-K, 1-C infusion | 1.81 ± 0.41 | 1.09 ± 0.11 |
| | n = 6 | n = 6 |

Mean ± SEM
* $p < 0.05$ vs sham and ANF-infused
** $p < 0.05$ vs sham

In the present experiments we show that chronic intravenous administration of ANF gradually reduced blood pressure to normal levels in the 2-K, 1-C hypertensive rats. This normalization in blood pressure was not accompanied by an increased natriuresis or diuresis, which suggest again, as in our previous acute experiments, that the drop in blood pressure is not secondary to a contracted circulatory volume. Surprisingly, chronically administered ANF produced a reduction in diuresis and natriuresis in 2-K, 1-C hypertensive rats which reached the levels shown by the normotensive group (Fig. 2). Both hypertensive groups presented, before treatment, higher natriuresis, diuresis and water intake than normotensive rats. This increase in water and sodium excretion can be explained by decreased sodium and water tubular reabsorption observed in animals with elevations of arterial blood pressure (15-17). Thus, ANF by reducing blood pressure would reduce pressure natriuresis and diuresis; water intake would follow diuretic changes.

PRA is significantly higher in not treated 2-K, 1-C rats than in sham-operated animals as it has been described in this experimental model at this stage of development of hypertension (18).

As seen in Table I, PRA, is much lower in ANF-infused than in not-infused 2-K, 1-C hypertensive rats and not different from that of sham-operated normotensive animals.

An explanation for the lower PRA in ANF-infused animals could be a direct inhibitory effect of ANF on renin production. Our study, however, does not provide any evidence for such an hypothesis.

A more attractive hypothesis would be that in lowering blood pressure ANF could break the circle of high blood pressure stimulating natriuresis with a negative sodium balance, which, in turn, would stimulate renin production (18).

It is well known that reduction in renal blood flow even in the presence of a non functional macula densa stimulates renin production (19,20). In the 2-K, 1-C hypertension model it is the clipped kidney which has a decreased renal blood flow and therefore an increased renin production. Borenstein *et al.* (21) have recently shown that the injection of atrial extracts increased total and medullary renal blood flow. It could be then theoretically possible that the injection of ANF by increasing renal blood flow in the clipped kidney could decrease renin production. However, as the ischemic renal kidney is fitted with a rigid clamp, the possibility of increased renal flow after ANF administration seems very unlikely.

It has been previously demonstrated that ANF relaxes pre-contracted vessels whatever the agent used to induce a contraction (10,12). Acute administration of ANF in renal hypertensive rats, either the two-kidney, one-clip or the one-kidney, one-clip model reduced blood pressure to normal levels (13), suggesting that ANF under those particular experimental circumstances was acting as a non specific vasodilator. In our present chronic experiments in which a normalization of both blood pressure and PRA was observed, the possibility that the decrease in the latter may play a partial role in lowering blood pressure cannot be excluded.


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Received September 5, 1984.
P.S.E.B.M. 1985, Vol. 178.
Accepted October 26, 1984.