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RAPID COMMUNICATIONS

REDUCTION OF BLOOD PRESSURE AND INCREASED DIURESIS AND NATRIURESIS DURING CHRONIC INFUSION OF ATRIAL NATRIURETIC FACTOR (ANF Arg 101-Tyr 126) IN CONSCIOUS ONE-KIDNEY, ONE-CLIP HYPERTENSIVE RATS

RAUL GARCIA, JOLANTA GUTKOWSKA, JACQUES GENEST, MARC CANTIN AND GAÉTAN THIBAULT

Clinical Research Institute of Montreal
110 Pine Avenue West, Montreal, Quebec, Canada H2W 1R7

Abstract. Conscious one-kidney, one-clip hypertensive rats and their normotensive controls were infused during 7 days with synthetic ANF (Arg 101-Tyr 126) at 100 ng/hr/rat (35 pmol/hr/rat) by means of osmotic minipumps. The basal blood pressure of 193 ± 6 mmHg gradually declined to 145 ± 6 mmHg at day 4 after the infusion was started. No changes in blood pressure were observed in ANF-infused normotensive rats. A significantly higher diuresis and natriuresis was observed in ANF-infused hypertensive rats when compared to the non-treated hypertensive group. No such changes were observed in ANF-treated normotensive animals. No differences in PRA were seen in any group. Atrial immunoreactive ANF was significantly lower in one-kidney, one-clip rats than in the normotensive animals, but whether this is the reflection of an increased release in the circulation remains to be elucidated. It is suggested that the hypotensive response of one-kidney, one-clip animals to ANF may be secondary to a dual mechanism, vasodilatation and volume depletion.

Introduction. We have recently demonstrated (1, 2) that chronic infusion of synthetic ANF (Arg 101-Tyr 126) reduces blood pressure in two models of experimental hypertension with probably different pathogenic mechanisms, but both with either a normal or decreased circulatory volume (3-5). However, a different pattern of sodium excretion was observed in them. Whereas two-kidney, one-clip (2-K, 1-C) hypertensive rats responded with a normalization of an initially higher pressure diuresis and natriuresis (1), no changes were observed in sodium excretion in ANF-infused SHR (2).

We have now chosen a low renin, presumably volume-expanded type (6) of experimental hypertension such as the one-kidney, one-clip (1-K, 1-C). Immunoreactive ANF has also been measured in atrial tissue.

Materials and Methods. One-kidney, one-clip hypertension was produced in male Sprague-Dawley rats (180-200 g) by constriction of the left renal artery with a silver clamp having a gap of 0.20 mm; one week later the contralateral kidney was removed. The normotensive control group was subjected to a sham-operation and one week later to a right nephrectomy.

Blood pressure was measured indirectly twice a week by means of a tail-cuff under light ether anesthesia as previously described (1). Once the blood pressure of 1-K, 1-C rats was 150 mmHg or higher during 4 consecutive weeks, the animals were put in individual metabolic cages for 3 to 4 days before the experiments were started to allow them to become accustomed to their new environment. The animals were kept on regular rat chow and tap

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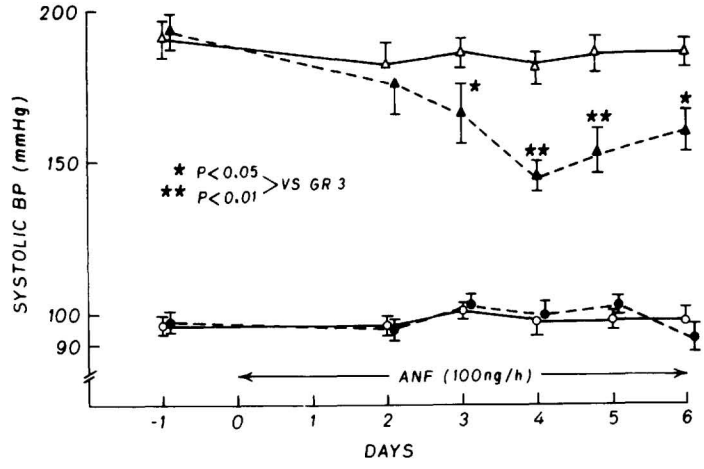

Figure 1 Effect of ANF (Arg 101-Tyr 126) infusion on blood pressure of 1-K, 1-C hypertensive and uninephrectomized animals.

water *ad libitum*. Forty-eight hours after this initial period the rats were separated in four experimental groups. Under light ether anesthesia, one group each of 1-K, 1-C and uninephrectomized rats, was implanted in the neck with osmotic minipumps (Model 2001, Alza, Palo Alto, CA) filled with synthetic ANF (Arg 101-Tyr 126) dissolved in 0.9% NaCl, and calculated to release 100 ng/hr (35 pmol/hr) of the peptide. The pumps were connected to the left jugular vein by a polyethylene catheter (PE-60). A second group each of 1-K, 1-C and uninephrectomized rats was also anesthetized and a piece of plastic tubing of the same size as the minipumps, was implanted subcutaneously. The left jugular vein was cannulated with a blind PE-60 catheter.

Urinary volume, water intake and indirect blood pressure were measured daily. Body weight was taken everyday or every other days. Urinary sodium was measured by flame photometry.

Seven days after the pumps were installed, the animals were decapitated, blood collected, heart excised and the atria carefully dissected.

Plasma renin activity was measured by radioimmunoassay of generated angiotensin I (7).

Both atria were processed simultaneously for each animal as previously described (8). Briefly, atria were homogenized in 2 ml of 0.1 M acetic acid for 1 min and centrifuged for 20 min at 30,000 rpm. The supernatant was stored at -70°C, then thawed and centrifuged for a second time. The pellet was discarded and the supernatant kept at -70°C until assayed. Atrial natriuretic factor was then measured by radioimmunoassay (8). Protein was measured by a modification of Bradford's method (9).

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

Results. There was no difference in the initial blood pressure (BP) between 1-K, 1-C and 1-K, 1-C + ANF-

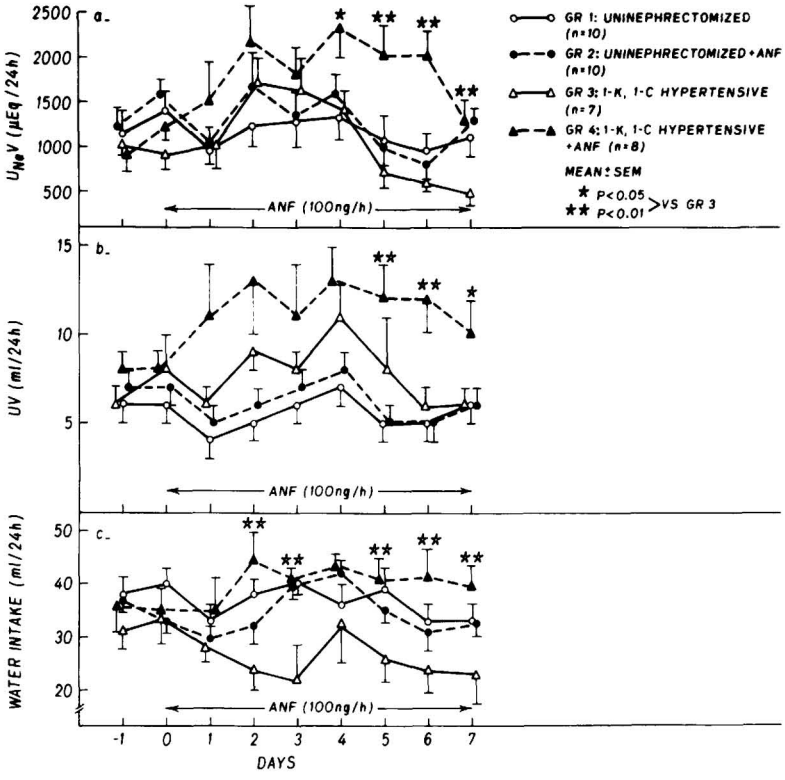

Figure 2 Effect of ANF (Arg 101-Tyr 126) infusion on natriuresis (a), diuresis (b) and water intake (c) in 1-K, 1-C hypertensive and uninephrectomized animals.

treated hypertensive rats, which was 191 ± 6 mmHg and 193 ± 6 mmHg respectively. The initial BP was also not different in the normotensive controls; 96 ± 2 mmHg in the uninephrectomized and 97 ± 3 mmHg in the uninephrectomized ANF-treated rats.

At day 3 after the infusion of ANF was started, a significant decrease ($p < 0.05$) was observed in the BP of 1-K, 1-C + ANF group, reaching the maximum decline at day 4, when the BP in non-infused and ANF-infused hypertensive animals was 181 ± 5 mmHg and 145 ± 6 mmHg, respectively ($p < 0.01$) (Fig. 1). At days 5 and 6 the BP was still significantly lower in the ANF-treated 1-K, 1-C group. No differences were observed between ANF-infused and non-infused normotensive controls. Sodium excretion, urinary volume and water intake in all experimental groups are depicted in Figure 2. No difference was seen in basal sodium excretion (Fig. 2a) between normotensive and hypertensive groups. However, a gradual increase in urinary sodium excretion was observed in the ANF-infused 1-K, 1-C group, reaching significantly higher values than basal excretion at day 2 ($p < 0.05$), and significantly different from the non-infused hypertensive group at days 4, 5, 6 and 7. No differences were observed in sodium excretion between ANF-infused and non-infused normotensive animals. A tendency to a decrease in sodium excretion was observed in the non-treated hypertensive rats during days 5, 6 and 7, however, those values were not significantly different from the basal ones. Urinary volume (Fig. 2b) closely followed sodium

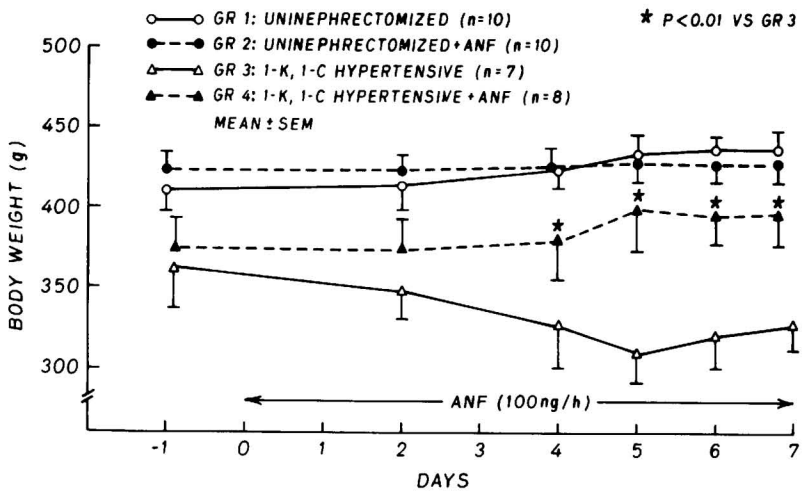

Figure 3 Effect of ANF (Arg 101-Tyr 126) infusion on body weight of 1-K, 1-C hypertensive and uninephrectomized animals.

excretion. At day 4, urinary volume was significantly higher in ANF-infused hypertensive rats than its basal value ($p < 0.05$) and at days 5, 6 and 7 significantly higher than in non-treated hypertensive rats. Again, no differences were observed between infused and non-infused normotensive animals. Starting from day 2, water intake (Fig. 2c), was also significantly higher in treated than in non-treated hypertensive rats.

Basal body weight after 4 weeks of hypertension (Fig. 3) was significantly higher in normotensive than in hypertensive groups ($p < 0.01$). No difference was observed between either both normotensive or both hypertensive groups. However, from day 4 and until the end of the observation period, the weight of ANF-infused hypertensive animals was significantly higher than in non-infused hypertensive animals. No difference was observed during the experiment between ANF-infused and non-infused normotensive rats.

PRA (ng AI/ml/hr) was not significantly different in any group: 1.14 ± 0.30 in uninephrectomized, 1.05 ± 0.27 in ANF-infused uninephrectomized, 3.96 ± 1.54 in 1-K, 1-C and 2.18 ± 1.09 in ANF-infused 1-K, 1-C rats.

Since no difference was observed in atrial immunoreactive ANF between infused and non-infused rats, the results of both hypertensive and both normotensive groups were pooled together.

As can be seen in Table I the total atrial content and concentration of ANF (expressed as μg/mg protein) are significantly lower ($p < 0.005$) in 1-K, 1-C than in uninephrectomized rats.

Discussion. The mechanism by which high blood pressure is sustained in the 1-K, 1-C model of hypertension is still under dispute. In the early stages it seems to be renin-dependent (10-12). In the chronic phases, 1-K, 1-C hypertension has been associated with an increase of exchangeable sodium (13) and a positive sodium balance (14). However, not every 1-K, 1-C hypertensive rat developed a positive sodium balance (14). In spite of the controversies there are strong evidences supporting the idea that this model of hypertension is a volume-dependent one, since an increased plasmatic volume has been found in the chronic stage, 60 days after surgery (6). Furthermore, permanent reversal of hypertension after the ischemic kidney is unclipped, is accompanied by a marked diuresis and natriuresis (15).

Table I

ATRIAL IMMUNOREACTIVE ANF IN 1-K, 1-C HYPERTENSIVE RATS

Group	ANF μg/atria	ANF μg/mg · protein
Uninephrectomized n = 20	14.47 ± 0.88	5.91 ± 0.38
1-K, 1-C n = 13	10.33 ± 1.33 *	3.40 ± 0.46 *

Values are means ± SEM

* $p < 0.005$ vs. control

Our present experiments show that chronic infusion of ANF in conscious 1-K, 1-C hypertensive rats produces an effect which is qualitatively similar to that induced when the ischemic kidney is unclipped, i.e., a decrease in BP and an increase in diuresis and natriuresis.

We have previously shown (1, 2) that chronic infusion of ANF in 2-K, 1-C and SHR, which have either a normal or low circulatory volume (3-5), produced a decrease of pressure natriuresis in the former and no change in sodium excretion in the latter. By contrast, the 1-K, 1-C, with a presumable increased plasma volume, responded to chronic infusion of ANF with diuresis and natriuresis. No such effect was observed in the ANF-infused normotensive rats. These findings suggest that the natriuretic response to chronically administered ANF could be determined by the state of the circulatory volume.

Both SHR (2) and 1-K, 1-C, but not 2-K, 1-C rats (16) have a significantly lower atrial immunoreactive ANF concentration than their respective normotensive controls. However, the hypotensive effect of ANF was much more marked in the SHR, whose blood pressure returned to normal levels, than in the present experiments. Those results suggest that plasma concentration of ANF may be low in SHR making these animals more sensitive to the infused peptide. In the 1-K, 1-C rat, by contrast, the lower concentration of atrial ANF could be the reflection of an increased release into circulation rising its plasma concentration and decreasing the vascular sensitivity to the peptide. This hypothesis is supported by recent *in vitro* experiments in which a decrease of ANF binding to mesenteric artery smooth muscle cells was found in 1-K, 1-C rats, suggesting a down-regulation of ANF vascular receptors (Schiffrin *et al.*, personal communication).

We have previously demonstrated (17) that ANF administration in conscious rats induced not only an increase in renal blood flow, but also in total splanchnic blood flow. This splanchnic vasodilatation may account for the hypotensive response we have observed in SHR and 2-K, 1-C rats, in which an increase in diuresis and natriuresis was not observed (1, 2). However, since 1-K, 1-C rats responded to ANF infusion with an increase in diuresis and natriuresis, it is possible that in this model of hypertension, the reduction of blood pressure induced by ANF is produced by a dual mechanism, vasodilatation and volume depletion.

No differences were seen in PRA between either normotensive and hypertensive or infused and non-infused animals. In contrast, in a renin-dependent model of hypertension as the 2-K, 1-C, chronic infusion of ANF normalizes both PRA and blood pressure (1). The different patterns of sodium excretion during chronic ANF administration observed in different models of hypertension in the rat, such as the

SHR (2), 2-K, 1-C (1), and 1-K, 1-C, raises the question whether ANF decreases blood pressure by different mechanisms depending of the pathogenic causes of high blood pressure.

In a previous publication (17), we have discussed the role of an increased renal blood flow in the natriuretic effect of ANF. In the present experiments it could be possible to explain the chronic natriuresis by the same mechanism. Since the ischemic kidney in 1-K, 1-C rats is fitted with a rigid clamp, the possibility of an increased renal blood flow during ANF administration seems, however, very unlikely. If that is true, we have to accept that the natriuresis produced during chronic administration of ANF in 1-K, 1-C rats is secondary either to a direct effect of the peptide on sodium tubular reabsorption or to a still unknown mechanism.

In spite of an increased natriuresis and diuresis, ANF-infused 1-K, 1-C rats gained weight (Fig. 3), whereas the non-infused hypertensive animals showed a progressive decline in body weight. This finding could be explained by a smaller food intake and increased catabolism. The same is also possible for water intake (Fig. 2).

It is tempting to speculate that the lower concentration of ANF in the atria of 1-K, 1-C rats reflects an increased release into the circulation triggered by an increased circulatory volume and an increased intra-auricular pressure, and intended to function as a compensatory mechanism preventing further increments in blood pressure and plasma volume.

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