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ATRIAL NATRIURETIC FACTOR INHIBITS THE SYMPATHETIC NERVOUS ACTIVITY IN ONE-KIDNEY, ONE-CLIP HYPERTENSION IN THE RAT

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Abstract. The one-kidney, one-clip model of rat hypertension was found to have an increased natriuresis following chronic infusion of atrial natriuretic factor (ANF). We have now found that this natriuretic effect of ANF is associated with a suppression of the initially elevated urinary excretion of norepinephrine and epinephrine and increase of the excretion of the main dopamine metabolite-dihydroxyphenylacetic acid as well as of the urinary dopamine to norepinephrine ratio. These data are compatible with the hypothesis that ANF suppresses the increased sympathetic activity in this model of hypertension and this action combined with opposite changes of dopamine may contribute to the natriuretic effect of ANF.

Introduction. The ability of chronic infusion of atrial natriuretic factor (ANF) to increase natriuresis and decrease arterial blood pressure have been studied in Goldblatt and genetic models of experimental hypertension (1, 2, 3). One-kidney, one-clip (1K-1C) hypertension in the rat was the only one, which exhibited an enhanced natriuresis and diuresis associated with a decline in the blood pressure (3). There is experimental evidence of increased sympathetic tone in 1K-1C rats, supported by an elevated plasma norepinephrine (NE), (4) NE turnover and its urinary excretion as well as dopaminergic activity disproportionately low relative to NE in these animals (5). The abnormalities in sodium balance found in 1K-1C (6) may thus be dependent on the excessive renal sympathetic tone in the clipped remaining kidney (7, 8) and causally linked to the development and/or maintenance of hypertension. The inhibition of the increased sympathetic nervous activity by ANF may also explain the natriuretic action of ANF in this not angiotensin-dependent model of hypertension in which the renin-angiotensin system was unaffected by ANF administration (3). The finding that ANF infusion resulted in a decrease of NE and increase of dihydroxyphenylacetic acid (DOPAC), the main dopamine (DA) metabolite in the rat suggests an inhibitory action of ANF on the sympathetic nervous activity which may have contributed to the natriuretic and diuretic action of ANF in 1K-1C rats.

Methods. Male Sprague-Dawley rats weighing 180-200 g underwent a protocol, previously described (3). Briefly, hypertension was produced by a constriction of the left renal artery with a silver clamp having a gap of 0.2 mm; one week later the contralateral kidney was removed. The control rats underwent an unilateral nephrectomy. Once the blood pressure was at least 150 mmHg during 4 consecutive weeks, the animals were kept in metabolic cages on regular rat chow and tap water ad libitum 3 to 4 days before the insertion of the miniosmotic pumps. The pumps filled with synthetic ANF (Arg 101-Tyr 126) to release 100 ng/h of the peptide for seven days, were implanted in the neck and connected to the left jugular vein with a catheter (PE-60); control animals were treated, as described (1). Each group averaged 7-10 rats.

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Urinary volume, water intake and blood pressure (BP) by the tail cuff method were measured daily. Urinary excretion of NE, epinephrine (E), DA, DOPAC, homovanillic acid (HVA) and creatinine were measured on the 7th day of continuous ANF administration, when the natriuretic action of ANF was evident.

Urinary NE, E and DA were determined radioenzymatically (9), urinary DOPAC and HVA by reverse-phase HPLC with electrochemical detection (10), sodium by flame photometry, creatinine colorimetrically.

Results are expressed as mean ± SEM. Statistical significance was calculated by Student's t-test for single comparisons and by one-way analysis of variance, Dunnett's and orthogonal Student's t-test for multiple comparisons.

Results. As seen in Table 1, urinary volume, sodium and creatinine excretion as well as water intake were higher in ANF infused 1K-1C than in non-treated 1K-1C animals. ANF administration was however without any effect on these parameters in sham operated rats. Blood pressure (Table 1) on the day prior to urinary collection was lower in ANF-infused 1K-1C than in 1K-1C rats whereas ANF did not affect the BP in uninephrectomized rats.

Baseline urinary NE and E excretions were significantly elevated in 1K-1C rats when compared to their one-kidney unclipped control in accordance with our previous observations (5) (Figure 1). The chronic infusion of ANF changed the excretory pattern of urinary catecholamines in 1K-1C rats, but left it unaffected in uninephrectomized rats. The ANF infusion caused a significant decrease in both NE and E excretion, normalizing their values. DA excretion moved however in opposite direction, but the difference in the mean DA excretion between sham infused (1.8 ± 0.4 µg/24 h) and ANF-infused 1K-1C (4.1 ± 1.4 µg/24 h) rats did not reach statistical significance; DA in uninephrectomized rats was 4.5 ± 1.3 µg/24 h and in ANF-treated uninephrectomized 5.1 ± 1.6 µg/24 h. When the DA/NE ratio was calculated, it showed a significant ANF-infusion-induced increase from 1.6 ± 0.3 to 4.7 ± 1.2, $p < 0.05$ in 1K-1C rats but not in uninephrectomized rats (from 5.18 ± 1.0 to 5.61 ± 1.2). The urinary DOPAC excretion was lower in 1K-1C rats (Figure 1) compared to sham operated rats and it increased in response to ANF infusion in 1K-1C rats, but did not change in uninephrectomized rats. HVA did not

TABLE 1
Effect of ANF chronic infusion (7th day) on water intake, urinary volume, sodium and creatinine excretion and blood pressure in 1K-1C and uninephrectomized rats

	Urinary volume (ml 24 h)	Sodium excretion (mmol/24h)	Water intake (ml/24 h)	Creatinine excretion (mg/24 h)	Blood † pressure (mmHg)
UNINEPHRECTOMIZED (n = 10)	7.3±1.9	1.20±0.21	33±4	7.0±0.9	97±6
UNINEPHRECTOMIZED + ANF (n = 9)	6.1±0.9	1.29±0.15	33±2	6.8±0.9	91±6
1K-1C (n = 7)	5.1±1.3	0.48±0.11	23±7	4.9±0.5	185±5
1K-1C + ANF (n = 8)	9.9±1.8*	1.36±0.26**	38±4**	8.5±1.1*	169±10*

* $p < 0.05$, ** $p < 0.01$ VS 1K-1C.
† 6th day of ANF infusion.

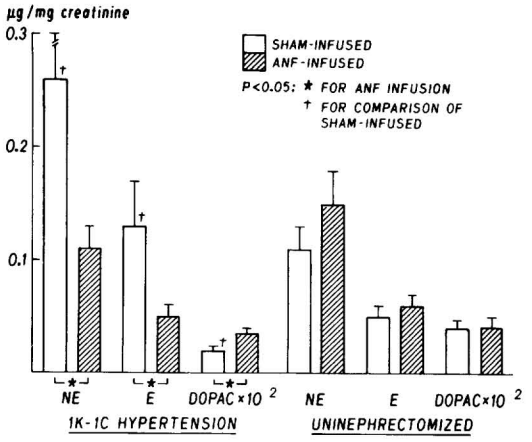

Figure 1: Urinary NE, E and DOPAC excretion at day seven of continuous infusion of ANF in 1K-1C and uninephrectomized rats.

change in response to ANF in any of the tested group: uninephrectomized sham infused rats 2.81 ± 0.15 µg/mg creatinine; ANF-infused 2.73 ± 0.5; 1K-1C sham infused 2.61 ± 0.29 and ANF-infused 2.77 ± 0.10.

Discussion. A long-term infusion of ANF increased the natriuresis and diuresis while the arterial blood pressure was decreasing only in the 1K-1C rats (3). Our data show that the ANF induced enhanced urinary volume and sodium excretion in 1K-1C animals is associated with a diminished urinary NE and E excretion and augmented DOPAC excretion. The urinary measurements of catecholamines (CA) are not an ideal way to monitor the sympathetic nervous activity. In the presence of a great variability in rat plasma CA we are, however, when testing cumulative actions of ANF, left with these measurements as the best alternative to determine the overall change of the sympathetic nervous activity over a certain period of time (11). Care must be taken to correct the urinary excretion of CA and their metabolites for renal excretory mechanisms by creatinine measurements. Urinary excretions of NE, E and DOPAC so corrected suggest that ANF induces in 1K-1C rats a decrease in noradrenergic as well as adrenergic and possibly also an increase in dopaminergic activity. On the other hand, ANF was without any influence on CA excretion in sham operated rats having apparently normal baseline sympathomedullary activity. The action of ANF inhibiting the sympathetic nervous activity may thus be present only when the baseline sympathetic tone is elevated. DA is considered to be a natriuretic compound while NE has antinatriuretic activity (7, 12). The ANF-induced increase in the ratio of urinary DA to NE might thus have been implicated in the mechanism of natriuretic action of this peptide in 1K-1C hypertensive rats.

The mechanism through which ANF exerts its natriuretic effect appears to be a combination of renal hemodynamic (and probably also a yet poorly defined tubular) actions (13) which strikingly resembles that demonstrated for DA (14). The finding that the action of ANF is blunted in animals pretreated with dopaminergic receptor antagonists (15) is compatible with an ANF-induced increase in kidney DA production or release to be related to the natriuresis. In contrast, NE acts as an antinatriuretic factor through the decrease in GFR and increase in tubular sodium reabsorption (7). Thus, the ANF-induced decreased noradrenergic activity could also potentiate the natriuretic and diuretic action of this peptide in 1K-1C animals. It remains to be established why ANF increased the creatinine excretion in rats with the clips on their renal arteries while the ANF action on creatinine in the unclipped kidney was absent. It is conceivable that the ANF infusion increases the GFR (13) at certain dose levels when its baseline value is decreased and it has no effect on the normal GFR which can be expected in the unclipped kidney.

The mechanism by which ANF affects the catecholaminergic system remains obscure. A chronic ANF infusion was able to suppress the compensatory increase in NE excretion following adrenalectomy (16). ANF also inhibits the NE production and stimulates the DA production in cultured human pheochromocytoma cells (unpublished observation). These findings and the present data suggest an inhibitory action of ANF on the dopamine-β-hydroxylase, the

enzyme determining the DA to NE ratio. Alternatively, ANF could also affect the cardiac sensory receptors and via a neuronal reflex decrease selectively the sympathetic tone in the renal nerves (17). This could be reflected by a decreased NE excretion since activation of these receptors results in a diminished NE release (18).

In summary, the chronic ANF infusion changed the excretory pattern of CA in a sense suggestive of a decreased noradrenergic and increased dopaminergic activity in 1K-1C rats while lowering the blood pressure and enhancing natriuresis and diuresis. Since both DA and NE are involved in renal water and sodium handling, these changes may have contributed to the natriuretic action of ANF in 1K-1C hypertension. This action of ANF appears to be limited to conditions in which the sympathetic nervous activity is increased.

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