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RAPID COMMUNICATION

CHRONIC INFUSION OF LOW DOSES OF ATRIAL NATRIURETIC FACTOR (ANF Arg 101-Tyr 126) REDUCES BLOOD PRESSURE IN CONSCIOUS SHR WITHOUT APPARENT CHANGES IN SODIUM EXCRETION

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Abstract. Conscious SHR and WKY rats were infused during 7 days with synthetic ANF (Arg 101-Tyr 126), 100 ng/hr/rat (35 pmol/hr/rat) by means of miniosmotic pumps. The SHR initial blood pressure of 177 ± 5 mmHg gradually dropped to 133 ± 3 and 142 ± 4 mmHg the last two days of infusion. No significant change in blood pressure was observed in the ANF-infused WKY group. No apparent difference in natriuresis or diuresis was observed in ANF-infused SHR and WKY when compared with non-infused control groups. A slight but significant lower immunoreactive ANF concentration was found in the atria of SHR than in their normotensive controls. No difference in cardiac weight was found between infused and non-infused rats. It is suggested that the hypotensive response observed in SHR and not in WKY is due to a decrease in vascular peripheral resistance. Whether ANF is involved in the development and maintenance of high blood pressure in SHR remains to be elucidated.

Introduction. We have recently demonstrated (1) that chronic infusion of synthetic ANF 101-126 (previously called ANF 8-33), which shares similar natriuretic and vasorelaxant activity with a native form (2, 3), reduced blood pressure to normotensive levels. In addition to its chronic hypotensive effect, ANF reduced pressure diuresis and natriuresis and normalized PRA in the 2-K, 1-C rat.

We have now chosen a non renin-dependent model of hypertension, SHR, in which it has already been suggested that ANF may play a pathogenic role (4).

The atrial content of immunoreactive ANF has also been measured.

Materials and Methods. Spontaneously hypertensive rats (SHR), 14-15 weeks old, and their normotensive Wistar-Kyoto controls (WKY), 14-15 weeks old, were purchased (Taconic Farms, Germantown, NY). Systolic blood pressure was measured indirectly by means of the tail cuff method (Narco Biosystems Inc., Texas, USA) in conscious, pre-warmed (37°C for 10 min) rats. The animals were accommodated in metabolic cages 3 to 4 days before the experiments were started and were kept on regular rat chow and tap water *ad libitum*. Forty-eight hours after this initial period the animals were separated in four experimental groups. Under light ether anesthesia, one group each of SHR and WKY rats, was implanted subcutaneously in the neck with osmotic minipumps (model 2001, Alza, Palo Alto, CA) filled with synthetic ANF 101-126 to release 100 ng/hr (35 pmol/hr) of the peptide. The pumps were connected to the left jugular vein by means of a polyethylene catheter (PE-60). A second group each of SHR and WKY, was equally anesthetized and a piece of plastic tubing with the same size as

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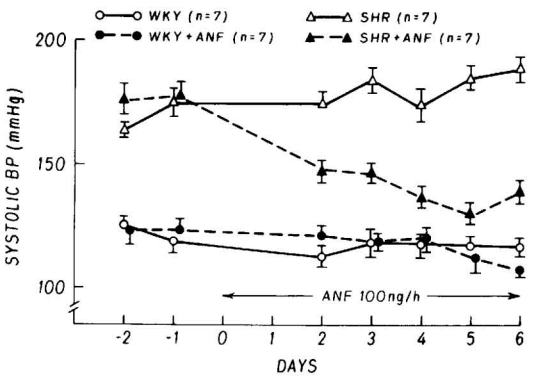

Figure 1: Effect of ANF 101-126 infusion on blood pressure of the SHR and WKY rats.

the minipumps were 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 at days -2, 2 and 7. Urinary sodium and potassium were measured daily in a flame photometer. On day seven, after the pumps were installed, the animals were anesthetized (i.p. pentobarbital, 50 mg/kg) the heart excised and weighed, and the atria were carefully dissected. Both atria were processed simultaneously for each animal as previously described (5). 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. The radioimmunoassay procedures have been described elsewhere (5). Protein content was measured by a modification of the method of Bradford (6). Some of the atria from each group have been preserved for histological studies. Results are expressed as means ± SEM. Single comparisons were done by the unpaired Student's "t" test. Analysis of covariance and the Scheffe's test were used for multiple comparisons.

Results. The initial BP was not significantly different between SHR and SHR + ANF, being 164 ± 2 mmHg for the former and 177 ± 5 mmHg for the latter. The initial BP was also no different in the normotensive groups, being 125 ± 3 mmHg in WKY and 124 ± 4 mmHg in WKY + ANF rats. As seen in Fig. 1 SHR infused with ANF gradually decreased their BP to normotensive levels no different from non-infused WKY rats. This decline in BP started early being significantly lower ($p < 0.05$) than non-infused SHR, 48 hr after the ANF infusing pumps were installed. On the last two days of the experiment, the mean systolic BP was 186 ± 4 mmHg and 189 ± 5 mmHg for SHR and 133 ± 3 mmHg and 142 ± 4 mmHg for the ANF-infused SHR ($p < 0.01$). In Fig. 2, sodium excretion, urinary volume and water intake in all four experimental groups are depicted. A significant higher natriuresis (Fig. 2a) and diuresis (Fig. 2b) were observed in both, ANF-infused and non-infused SHR than in WKY rats ($p < 0.01$). However, ANF infusion did not modify this pattern of sodium and water excretion either in hypertensive or normotensive groups. Body weight was not significantly different between ANF-infused and non-infused SHR, being at day -2, 263 ± 2 g and 263 ± 3 g, at day 2, 266 ± 2 g and 267 ± 4 g, and at day 7, 270 ± 4 g and 272 ± 6 g, respectively. For WKY rats the weight was 279 ± 13 g and 304 ± 10 g at day -2, 293 ± 13 g and 314 ± 9 g at day 2, and 313 ± 15 g and 325 ± 8 g at day 7, for infused and non-infused animals, respectively. Those differences were not significant.

Since no significant difference in the atrial content of immunoreative ANF was observed between ANF-infused and

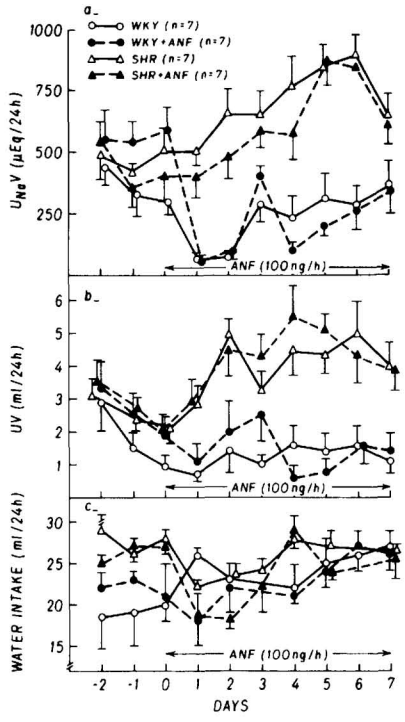

Figure 2: Effect of ANF 101-126 infusion on natriuresis (**a**), diuresis (**b**) and water intake (**c**) in SHR and WKY rats.

non-infused rats, the results from both SHR and both WKY groups were pooled.

As seen in Table I total content of immunoreactive ANF is significantly lower in SHR than in WKY rats. However, it would be necessary to emphasize that their lower concentration could be due to the fact that in ANF-infused SHR, the actual concentration of ANF is lower though not significantly so, than in non-infused rats. This difference however, is lost when the results are expressed per mg of protein. Heart weight was significantly higher ($p < 0.05$) in SHR, 1083 ± 18 mg, and in ANF-infused SHR, 1078 ± 46 mg, than in WKY, 925 ± 23 mg and than in ANF-infused WKY, 873 ± 23 mg. No differences were observed between infused and non-infused groups.

Hematocrit was slightly but significantly higher ($p < 0.01$) in ANF-infused SHR, 47 ± 1, than in SHR, 44 ± 1. No differences were observed in the normotensive controls, being 46 ± 1 and 43 ± 1 for WKY and ANF-infused WKY rats, respectively.

Discussion. It is well known that an elevated peripheral resistance with vascular structural changes is often associated with high blood pressure in the SHR (7); furthermore, an increased sensitivity of the mesenteric vessels to norepinephrine before the development of hypertension has been described (8). The origin of those vascular changes is uncertain, but it is generally accepted that the peripheral renin-angiotensin system is not involved (9-11). Sonnenberg *et al.* (4) have recently found that the injection of atrial extracts from SHR produced less natriuresis than those from WKY rats. They suggested that the decrease in natriuretic activity could be associated with an increased blood concentration of ANF, which, somehow, could

Table I

ATRIAL IMMUNOREACTIVE ANF IN SHR AND WKY RATS

Group	ANF μg/atria	ANF μg/mg protein
SHR (n = 10)	5.03 ± 0.23 [a]	1.21 ± 0.32
WKY (n = 8)	6.16 ± 0.42	1.47 ± 0.10

Values are means ± SEM
a: $p < 0.025$ vs control

be involved in the pathogenesis and maintenance of high blood pressure in this model of hypertension. In the present work a lower concentration of atrial immunoreactive ANF in SHR has been found, however, because of the reasons given in the results section, this difference need to be confirmed with a larger group of animals. Though statistically significant the biological relevance of this slight difference remains to be elucidated. A word of caution should be put forward when comparing results obtained with different methods. In our experiments immunoreactive atrial ANF has been measured using antibodies raised against ANF 101-126, which presents low cross-reactivity with longer forms (5). It is not well known whether the storage form of ANF is either a long or short peptide or rather a mixture of peptides differing in length. By measuring immunoreactive ANF we may be looking for the shorter peptide, while if injected *in vivo*, the longer form could theoretically be converted to the more active shorter form. It has been already demonstrated that the length of the amino acid chain is important in the natriuretic and relaxant activities of ANF (12, 13).

The fact that in SHR the blood pressure decreased to levels no different than their normotensive controls after being infused with doses of ANF ten times lower than those used in the 2-K, 1-C rats (1), suggests that SHR have either low or normal plasma levels of ANF. For a rat of 250 g body weight, with a blood volume equivalent to 50 ml/kg body weight, the amount of ANF delivered per minute would achieve a concentration of approximately 135 pg/ml blood, which is roughly equivalent to that found by radioimmunoassay in normal rats (14; Gutkowska *et al.*, unpublished results). A decrease in plasma levels of ANF in SHR could explain the increased sensitivity of these rats to norepinephrine (8), since ANF not only relaxes norepinephrine pre-contracted vascular strips (15, 16) but also shifts the norepinephrine dose-response curve to the right, this effect being more remarkable at low doses of the vasoconstrictor (2, 13). Since ANF infusion did not produce any apparent change in natriuresis and diuresis in either SHR or WKY rats, we may assume that the hypotensive response is not due to a circulatory volume contraction. However, since we have not performed balance studies, i.e. sodium intake versus sodium excretion, this data need to be confirmed. The slightly higher hematocrit in ANF-infused SHR suggests a certain degree of water loss. However, because no apparent differences were found in either natriuresis, urinary volume or weight between ANF-infused and non-infused groups, that possibility seems not to be very likely. Furthermore, the quantity of ANF necessary to induce a natriuretic response when administered as a bolus is much higher than the total amount injected during one minute in the present experiments (3). A definite answer to whether or not the chronic hypotensive effect of ANF observed in SHR is associated with a contracted circulatory volume should be given by measuring extracellular fluid volume under the same experimental conditions described in the present work. Acute administration of ANF (101-126) in conscious rats is known to produce an important renal and splanchnic vasodilatation without modifications of cardiac output (17). Thus, a decrease in total peripheral resistance without modification of cardiac output may well explain the hypotensive effect of ANF, at least in acute experiments. Whether the same changes could be involved in chronic experiments is not known. Because of its known effects in preventing or inhibiting the effect of norepinephrine (2, 13, 15, 16), ANF could reset the increased sympathetic nervous activity knwon to be present in SHR (18) to normal levels and thus normalize vascular peripheral resistance. On the other hand, the lack of hypotensive response in the WKY normotensive rats could be due to the lack of an increased sympathetic nervous activity in those animals (19).

We have previously shown (1), that chronic ANF infusion in 2-K, 1-C hypertensive rats produced a partial reduction in cardiac hypertrophy. Such was not the case in the present experiments, in which no difference in cardiac weight was observed in ANF-infused and non-infused SHR. This

observed difference between 2-K, 1-C and SHR could be secondary to either a different pathogenetic mechanism, or a different duration. Cardiac hypertrophy is secondary to the rise in blood pressure in 2-K, 1-C rats, whereas it may be found before the development of significant hypertension in the SHR (20).

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