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BODY FLUIDS AND PLASMA ATRIAL PEPTIDE AFTER ITS CHRONIC INFUSION IN HYPERTENSIVE RATS

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ABSTRACT. To determine the role of body fluid volume in the chronic hypotensive effect of atrial natriuretic factor (ANF), spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats were infused with the peptide (Arg 101 - Tyr 126) at a rate of 100 ng/h/rat for 5 days. Blood pressure (BP) was decreased from 176 ± 4 to 133 ± 3 mmHg in the SHR group 4 days after ANF infusion was initiated, whereas no changes were observed in ANF-infused WKY animals. Starting 5 days after the infusion began, body fluid measurements revealed no differences in plasma, blood and extracellular fluid volumes or in interstitial spaces. BP and plasma ANF concentrations were determined in another set of experiments before, during and after chronic ANF infusion. BP declined from 169 ± 3 to 133 ± 5 mmHg in SHR 5 days after the infusion commenced, but returned to basal values by day 10 or 11. Plasma ANF was significantly higher in SHR than in WKY rats throughout the observation period. However, there were no discernible changes in this parameter in ANF-infused SHR compared to non-infused SHR. A 3-fold rise in plasma ANF was noted in infused WKY rats at day 3 only. It is concluded that the chronic hypotensive effect of ANF in hypertensive animals is not related to changes in either body fluid volume or distribution. Moreover, the finding that chronic ANF infusion reduces BP in SHR without altering its plasma levels suggests a rapid ANF turnover.

INTRODUCTION. We have recently demonstrated that chronic infusion of atrial natriuretic factor (ANF) decreased blood pressure (BP) in spontaneously-hypertensive rats (SHR) without apparent changes in sodium excretion (1). However, since ANF-infused SHR presented slightly higher hematocrit values than non-infused hypertensive controls, the reduction in BP could have been mediated via a contracted circulatory volume. A similar increase in hematocrit was reported when either atrial extracts were injected into normotensive rats (2) or synthetic ANF was administered to anesthetized dogs (3). The latter investigation indicated that the elevated hematocrit ratios could have been due to a shift of fluid out of the intravascular compartment. This hypothesis was further supported by the observation of increased hematocrit and reduced plasma volume after acute administration of ANF to nephrectomized rats (4). In order to clarify whether, in SHR, the hypotensive effect of chronically-administered ANF, was partially caused by a shift of body fluids, we studied body fluid volume after prolonged treatment with the synthetic atrial peptide. Since the plasma levels of ANF are known to rise in SHR (5, 6), we also investigated whether they change after chronic ANF infusion.

MATERIALS AND METHODS

Protocol #1 This study was performed on 14- to 15-week-old SHR and their

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age-matched normotensive Wistar-Kyoto (WKY) controls (Taconic Farms, Germantown, NY). The animals were conscious and pre-heated (37°C for 10 min) when their systolic BP was measured indirectly by the tail-cuff method (Narco Biosystems Inc., TX). They were then housed in individual metabolic cages for 3 to 4 days to enable them to adapt to their new environment, and were fed regular rat chow and tap water "ad libitum". Each strain was separated into 2 experimental groups 2 days after this adaptation period. Osmotic minipumps (Model 2001, Alza, Palo Alto, CA) calibrated to last for 7 days, were implanted in the neck, under light ether anesthesia, in one group each of the SHR and WKY rats. They contained synthetic ANF (Arg 101 - Tyr 126, Institut Armand Frappier, Laval, Que.) dissolved in 0.1 M acetic acid and volume-completed with 0.9% NaCl; release of the peptide was calculated at 100 ng/h (35 pmol/h). The pumps were connected to the left jugular vein by a polyethylene catheter (PE-60). The remaining SHR and WKY groups were likewise anesthetized, and a piece of plastic tubing, of the same size as the minipumps, was implanted s.c.; the left jugular vein was cannulated with a blind catheter. The day on which the pumps were installed corresponded to day 0.

Systolic BP and body weight were measured on days -1, 3 and 4. On day 5, the animals were anesthetized with sodium pentobarbital (60 mg/kg body weight i.p.) and right intra-carotid artery and intra-jugular vein catheters were installed. Twenty-four h later, when the animals were conscious, body fluid volume was determined by dye-dilution methods (7, 8). Plasma volume was estimated with Evans blue, and extracellular fluid volume by thiocyanate space. Twenty-five mg of thiocyanate contained in 0.20 ml of saline was injected through the venous catheter. Thirty-five min later, 0.2 ml of a solution containing 3 mg of Evans blue was administered by the same route. After each injection, the catheter was washed with 0.15 ml of saline. Five min after the Evans blue injection, 1.0 ml of blood was withdrawn through the arterial catheter and centrifuged. Evans blue and thiocyanate concentrations in the plasma samples were determined by spectrophotometry, and standard formulas were used to calculate the different volumes (7).

Protocol #2 SHR and WKY rats of the same age as in Protocol #1 were housed individually, separated into 4 groups (as described earlier) and equipped with osmotic minipumps calibrated to deliver the same amount of ANF during 7 days as mentioned before. Systolic BP was measured on days -4, 2, 4, 5, 7, 8, 10 and 11. Blood was withdrawn, on days 0, 3, 6, 9 and 12, under sodium pentobarbital anesthesia (60 mg/kg body weight i.p.), by jugular vein puncture, and the volume replaced with blood from donor rats.

The blood samples were collected in glass tubes containing the following protease inhibitors at final concentrations of: 1.0×10^{-5} M EDTA, 5×10^{-6} M pepstatin, 1,000 U/ml aprotinin and 3×10^{-5} M phenylmethylsulfonyl fluoride. The samples were immediately centrifuged at 3,000 rpm for 10 min at 6°C.

ANF was extracted from plasma with Vycor glass beads (Corning Glass Works, Corning, NY) and measured by RIA as described elsewhere (9). The detection limit of the assay is 0.75 pg/tube. The inter- and intra-assay coefficient of variance are below 14%.

The results are expressed as means ± SEM. The data presented in Figures 1 and 2, and the hematocrit values, were analyzed by two-way analysis of variance with repeated measures to globally test the time effect, the group effect and the group interaction by time. One-way analysis of variance was employed with repeated measures for each group to globally test the time effect. Dunnet's test was applied whenever a level of significance was found ($p < 0.05$). The data in Table 1 were analyzed by one-way analysis of variance.

RESULTS

Protocol #1 Figure 1 depicts the BP in all four groups before and after the pumps were installed. The initial BP values were not significantly different between either SHR and SHR+ANF or WKY and WKY+ANF. On days 2 and 4, ANF-infused SHR presented a significant decrease in BP ($p < 0.01$), with a maximum reduction of 44 ± 5 mmHg on day 4.

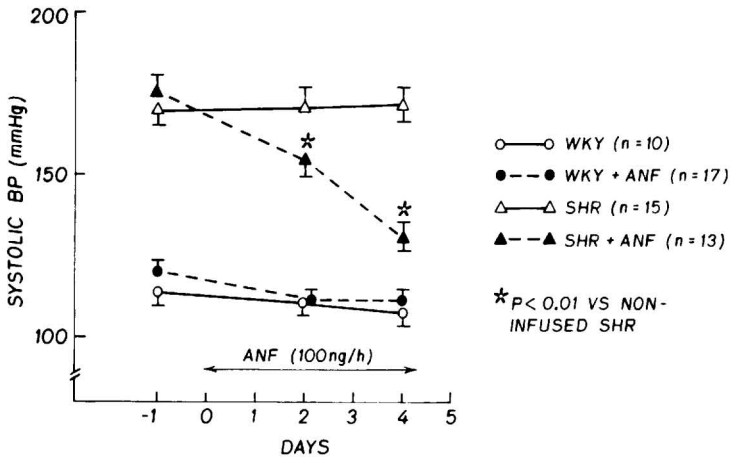

Figure 1 Effect of chronic infusion of ANF on BP in SHR and WKY rats.

No changes were observed in the other 3 groups.

As represented in Table 1, no differences were noted in body fluid volume between either SHR and WKY rats, ANF-infused or non-infused.

Protocol #2 BP was significantly lower in ANF-infused SHR in comparison to its basal values and those of non-infused SHR from days 2 to 8. On days 7 and 8, after the pumps were installed, there was a tendency for the BP to rise, reaching basal values on days 10 and 11 (Figure 2A). No significant changes were evident in the WKY groups.

The basal plasma ANF values were significantly higher in SHR than in WKY rats (Figure 2B). No significant fluctuations occurred in plasma ANF levels in either infused or non-infused SHR during the observation period. A different pattern was, however, seen

TABLE 1

BODY FLUID VOLUMES IN SHR AND WKY RATS AFTER CHRONIC INFUSION OF ANF (ARG 101 - TYR 126)

Group	Plasma volume (ml/100 g b.wt)	Blood volume (ml/100 g b.wt)	ECFV * (ml/100 g b.wt)	Interstitial space (ml/100 g b.wt)
WKY n=10	4.18 ± 0.55	6.94 ± 0.97	33.33 ± 2.29	25.72 ± 2.38
WKY+ANF n=17	5.08 ± 0.50	8.37 ± 0.77	33.77 ± 1.47	28.60 ± 1.60
SHR n=15	5.14 ± 0.58	8.61 ± 0.91	28.04 ± 1.49	24.82 ± 1.33
SHR+ANF n=13	5.65 ± 0.78	8.43 ± 1.27	32.20 ± 1.91	26.78 ± 1.99

Values are means ± SEM

* Extracellular fluid volume

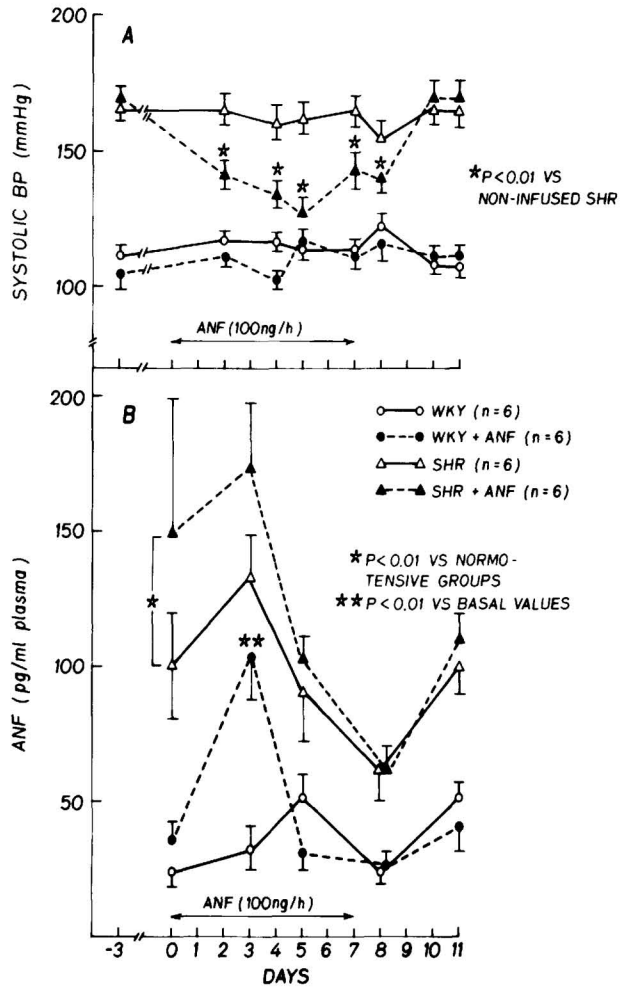

Figure 2 Effect of chronic infusion of ANF on (A) BP and (B) its plasma levels.

in normotensive WKY animals. On day 3, ANF-infused WKY rats presented a 3-fold increase in plasma ANF concentrations ($p < 0.01$), which returned to basal values (day 0) during the ensuing days.

There were no differences in hematocrit between either normotensive and hypertensive, ANF-infused or non-infused animals (results not shown).

DISCUSSION. We have previously reported (1) that chronic infusion of relatively low doses of ANF reduces BP in SHR without apparent changes in urinary sodium excretion, which indicates that the hypotensive effect is not secondary to a contraction of circulatory volume. However, this possibility cannot be completely excluded, since sodium excretion is not an accurate reflection of sodium balance. Futhermore, a contraction of blood volume could be the consequence not only of external losses of sodium and water but also of extravascular fluid shifts. These possibilities have been suggested by increased hematocrit values obtained after the acute administration of atrial extracts or synthetic ANF (2, 3).

The finding of a decreased plasma volume following the acute administration of ANF to nephrectomized rats (4) further upholds the likelihood of a contracted blood volume secondary to

inter-compartmental shifts of body fluids. However, our experiments demonstrated that this was not the case during chronic infusion of low doses of ANF. Body fluid distribution, similar to that reported in the literature, using comparable methods (7, 10, 11), was not different in ANF-infused and non-infused animals, even when the measurements were taken at a time when ANF produced significant hypotension in SHR. It is quite conceivable then that the response of body fluid distribution to acute or prolonged administration of ANF could be completely dissimilar. The present results confirmed our previous studies (1), in which we suggested that chronic infusion of ANF decreases BP in SHR without changes in sodium metabolism or body fluid distribution. The chronic hypotensive response could be affected by many known and probably several unknown functions which could be associated with the chronic administration of ANF. In our earlier report (1), we hypothesized that the chronic hypotensive effect of ANF could be secondary to a decrease in total peripheral resistance. However, since some investigators have reported that acute administration of ANF induces a decline in cardiac output (12), the latter could play a role in the chronic hypotensive action of the peptide. A fall in peripheral resistance to ANF has been reported in two-kidney, one-clip animals (13), but whether this is secondary to a direct effect of the peptide on smooth muscle or to an inhibition of renin activity (14, 15) is not known. Moreover, no information is yet available as to whether chronic administration of ANF modifies either vascular peripheral resistance or cardiac output.

In the second set of experiments, we reproduced our previous results (1) as well as those described above. Chronic infusion of low ANF doses decreased BP in SHR. Moreover, this hypotensive effect lasted as long as the minipumps were operant, with BP slowly returning to pre-infusion levels after day 7. Once again, ANF infusion did not modify BP in normotensive animals (Figure 2). We (5) and other investigators (6) have reported that plasma ANF concentrations are higher in SHR, when they reach the hypertensive stage, than in WKY rats. We have now further substantiated this finding. One unexpected observation derived from our present experiments was the similarity of plasma ANF levels in SHR, whether or not they were infused with the peptide. These results were in contrast with those in normotensive rats, in which a clear and significant, albeit transitory elevation of plasma ANF was noted in the infused group. The explanation for these apparently contradictory observations is not by any means clear. It has recently been reported that the disappearance time of ANF (Arg 101 - Tyr 126) from the circulation is less than 30 sec (16). Since ANF's rapid disappearance from the circulation seems not to be due to degradation in the blood (17) and high-affinity binding sites and internalization of ANF have been described in vascular smooth muscle (18, 19), it could be suggested that, when low doses of ANF are infused into the circulation of SHR, which already have high levels of plasma ANF, the peptide is rapidly captured by vascular receptors impeding a measurable rise in its plasma concentrations but not its physiological impact (hypotension). Since, however, we were able to detect, albeit transitory, changes in the plasma levels of the peptide in ANF-infused WKY rats, we may suggest that the turn-over of ANF in SHR and WKY rats may be different. A fast ANF turnover in SHR (i.e., increased synthesis accompanied by rapid disappearance) could explain both its high plasma concentrations and its undetectable changes when infused exogenously. The low content of atrial ANF in SHR (5, 6), suggesting its increased release, lends some support to our hypothesis.

We have previously stated (1) that ANF infusion in normovolemic WKY rats does not enhance sodium excretion even when, as is reported now, a rise in plasma ANF has been observed. This indicates that blood volume could be an important determinant in the natriuretic response to ANF (20, 21).

In summary, we have demonstrated that chronic infusion of relatively low doses of ANF into SHR decreases BP without a change in either its plasma level or body fluid volume. Our results suggest that the hypotensive effect could be secondary to a direct

action of the peptide either on peripheral vascular resistance or cardiac output.

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