Summary: We recently discovered a potent natriuretic factor in cardiac atrial tissue. The present experiments were designed to determine whether hypertension was associated with altered tissue content of this atrial natriuretic factor. Extracts were prepared using fresh atria from spontaneously hypertensive rats of the Okamoto strain and from their Wistar-Kyoto controls. Two groups of anesthetized, normovolemic rats (Sprague-Dawley) were used to measure the renal natriuretic and chloriuretic effect of each type of extract. Results indicate that atrial content of natriuretic factor is reduced in hypertensive rats compared to control animals. We speculate that chronic release of the factor could have depleted atrial stores, and that increased blood levels of atrial natriuretic factor may be involved in the generation and maintenance of hypertension in this model.

(Hypertension 5: 672-675, 1983)

Key Words • natriuretic hormones • spontaneous hypertension • kidney electrolyte excretion

Methods

Preparation of Atrial Natriuretic Factor

Spontaneously hypertensive rats (SHR, 300-325 g body weight, 16-17 weeks old, n = 65) and their normotensive Wistar-Kyoto controls (WKY, 300-325 g body weight, 16-17 weeks old, n = 65) were obtained simultaneously from Taconic Farms Inc, Germantown, New York. Three randomly chosen animals from each group were anesthetized with pentobarbital for measurement of femoral arterial blood pressure. Values were 160/120, 110/92, and 158/120, for SHR, and 106/93, 100/88, 106/92 mm Hg for WKY. Although the pressures in the hypertensive rats were lower than expected, it is known that pentobarbital lowers blood pressure in this model. The two groups of animals were processed within 2 days of each other. The rats were killed by cervical dislocation, and the hearts were removed immediately. After each heart was washed in ice-cold sodium phosphate-buffered saline (pH = 7.2), the atria were dissected, blotted lightly, and chopped with scissors. Subjectively, atria from SHR appeared small and shriveled compared to those from WKY. Total wet weights of atrial tissue from the 65 animals were 2.95 g (SHR) and 3.86 g (WKY).

Extraction of atrial natriuretic factor from pooled tissue of each group was according to the method of deBold. Five volumes of 1 M acetic acid with stabilizers (1 mg/liter pepstatin and 1 mg/liter phenylmethylsulfonyl fluoride) were added to each volume of tissue. The mixture was homogenized with a polytron for 3 seconds, maintained at 0° C for 1 hour, and then centrifuged for 15 minutes at 10,000 rpm and 4° C. The supernatant was saved and a further 2.5 volumes of acetic acid with stabilizers were added to each volume of tissue. The mixture was homogenized with a polytron for 3 seconds, maintained at 0° C for 1 hour, and then centrifuged for 15 minutes at 10,000 rpm and 4° C. The supernatant was saved and a further 2.5 volumes of acetic acid with stabilizers were added to the precipitate. The two were mixed vigorously and again maintained at 0° C and centrifuged. The resulting supernatant was pooled with that decanted from the previous centrifugation. The pH was adjusted to 7.6 with ammonium hydroxide, the pooled supernatants were centrifuged for 30 minutes at 30,000 rpm and 4° C, and the...
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The final supernatant was passed through a column of Bio-gel P2 (Biorad Products, Richmond, California). This column had been equilibrated with 1.0 M acetic acid. The bed volume was 650 ml and the volume of each collected fraction was 2 ml. These fractions were monitored for protein content by UV spectrophotometry at 280 nm, and for salt concentration by conductivity measurements. All fractions falling within the first protein peak were pooled and lyophilized. The freeze-dried product was reconstituted with 4 ml distilled water, left to incubate at 20°C for 2 to 3 hours, then centrifuged. The supernatant was divided into 0.1 ml aliquots, which were kept at —70°C until use. For bioassay, appropriate dilutions with isotonic saline were made for injection of extract equivalent to different numbers of atria (see Results). Each final injection volume was 0.15 ml.

Bioassay Procedure

Male Sprague-Dawley rats (body weight range = 231–309 g) were anesthetized with Inactin (10 mg/100 g body weight, intraperitoneally) and kept at a body temperature near 38°C. After tracheostomy, the left jugular vein was cannulated for constant infusion of Ringer solution (1 ml priming dose, followed by 1.2 ml/hr). A femoral artery and vein were cannulated for blood pressure measurement and injection of test substance, respectively. The bladder was exposed through a suprapubic incision and catheterized. An equilibration period of at least 30 minutes was allowed before urine was collected. After a 20-minute control period, atrial extract was injected intravenously and flushed into the circulation with 0.1 ml of saline within 1 minute. Four consecutive 5-minute urine collections and a further 20-minute postinjection period completed the experiment. Each animal was used for one bioassay. Four experiments were done per day: in the morning two operators simultaneously prepared one rat each, which received one of the two (coded) types of freshly diluted extracts. This procedure was repeated in the afternoon. The order of injection was varied sequentially between operators and from day to day, thus eliminating systematic bias. Urine volumes were measured by weighing, urine sodium and potassium concentrations by flame photometry, and urine chloride by electrometric titration. Excretory data were calculated per gram of kidney weight for each animal, and group means were expressed ± 1 se. Statistical comparison was by Student's t test.

Results

Atrial factor, equivalent to extract from one atrium of either normotensive (WKY) or hypertensive donors (SHR), was injected intravenously in 12 normal anesthetized rats each. Atrial equivalence was calculated from the number of hearts used and the dilution factor for reconstituted extract. The resulting absolute increases in sodium chloride excretion over the ensuing 10 minutes compared to the previous 20-minute control period were not significantly different (WKY = AU NaV = 3.89 ± 0.46; AU aV = 4.94 ± 0.57; SHR = AU NaV = 3.34 ± 0.48; AU aV = 4.70 ± 0.56 μEq/min/g kidney weight). These results show that atria from spontaneously hypertensive rats are not lacking in natriuretic factor. However, the dose given might have been supramaximal, obscuring possible differences in content between the two types of atria. Therefore, we obtained a dose-response curve using WKY atrial factor at different dilutions in groups of six bioassay rats each. Results (fig. 1) indicate that, indeed, extract from one atrium caused a maximal renal excretory response. A further two groups of 12 bioassay rats each were then used to measure the renal effect of WKY and SHR atrial factor at a dilution equivalent to one-third of one atrium. A significantly greater excretion of sodium chloride was found in rats injected with WKY factor (fig. 2), indicating that spontaneously hypertensive animals had less natriuretic factor in their atria than their normotensive controls.

The reduced response was not an artifact due to differences between the two groups of bioassay rats.
Figure 2. Renal excretion in two groups of normovolemic bioassay rats (n = 12 each) of (A) sodium and (B) chloride. Intravenous injection of atrial natriuretic factor (ANF) from SHR (broken lines) and WKY rats (solid lines) is indicated by the vertical arrow. Statistical significance of the difference in excretion is shown by * (p < 0.01).

(tables 1). Control arterial blood pressures, hematocrits, plasma electrolytes, and body and kidney weights were essentially identical. The decrease in blood pressure in the 10-minute period after injection of the extract was the same in both groups. Since sodium chloride excretions were significantly different, the data suggest that hypotensive and natriuretic effects of atrial extract are not directly related. However, injection of the higher concentration of extract (1 atrium equivalent) caused not only a greater natriuresis but also a larger fall in blood pressure, from 129 ± 3 to 112 ± 3 mm Hg (SHR extract), and from 127 ± 4 to 112 ± 4 mm Hg (WKY extract).

Discussion

The hypotensive effect of atrial extract suggests a simple explanation for blood pressure differences between SHR and WKY rats. However, neither the high nor the low dose of the two types of extract elicited differences in the cardiovascular response of bioassay rats, although the higher dose of each type was associated with more severe hypotension. Therefore, there appears to be no differential content of hypotensive factor in the two groups, pointing to the natriuretic action of atrial extract as a possible explanation for the difference in blood pressure between SHR and normotensive WKY.

Given the large number of atria harvested from both SHR and WKY groups, it is unlikely that a systematic difference in the proportion of total atrial tissue collected could account for the apparent difference in atrial natriuretic factor (ANF) content. However, it is possible that differences in morphology (including a reduction of total atrial tissue per hypertensive rat heart) might be responsible for the difference in extractable factor. The observation of reduced atrial content of natriuretic factor in SHR could then have two opposite explanations: either production of ANF by atrial cardiocytes (or the number of cardiocytes) might be reduced, or chronic release of ANF into the circulation might deplete tissue stores, analogous to chronic stimulation of vasopressin release. 7 In the former case, the difficulty of sodium elimination in genetically hypertensive rats 8 would be due to an inadequate stimulus affecting essentially normal kidneys, whereas in the

| Table 1. Comparative Data for Normal Bioassay Rats Before and After Injection of Low-Dose Atrial Extract from SHR and WKY rats |
| --- | --- | --- | --- | --- | --- |
| Rat | BP (mm Hg) | Hematocrit (%) | P<sub>Na</sub> (mEq/liter) | P<sub>K</sub> (mEq/liter) | BW (g) | KW (g) |
| SHR | Before | After | Before | After | Before | After | Before | After | Before | After |
| 124 ± 3 | 116 ± 4* | 45 ± 2 | 45 ± 2 | 137 ± 2 | 137 ± 2 | 4.3 ± 0.3 | 4.2 ± 0.4 | 272 ± 26 | 2.49 ± 0.20 |
| WKY | 124 ± 3 | 115 ± 3* | 45 ± 2 | 46 ± 2 | 137 ± 3 | 137 ± 2 | 4.6 ± 0.3 | 4.6 ± 0.3 | 270 ± 18 | 2.35 ± 0.21 |

Abbreviations: BP = arterial blood pressure; P<sub>Na</sub>, P<sub>K</sub> = plasma sodium and potassium concentrations; BW = body weight; KW = kidney weight.

*Significant difference, before-after; p < 0.001.
latter case, a primary defect in kidney function would be compensated by an increased stimulus for sodium excretion. Although our data do not allow discrimination between these alternatives, the second possibility is in agreement with DeWardener and MacGregor’s hypothesis on the etiology of hypertension, and is supported by the following indirect evidence: Specific atrial intracellular granules were increased in rats after chronic fluid deprivation, and decreased after saline and DOCA-loading. Since the natriuretic activity is found in the granular fraction, these results suggest that increased systemic release of ANF may be associated with reduced atrial tissue storage.

It should be emphasized that the ANF is different from the “natriuretic hormone” extracted from plasma, urine, or kidney tissue (for literature on natriuretic hormone, see refs. 3, 11). Natriuretic hormone seems to be an endogenous Na-K ATPase inhibitor, whereas ANF does not inhibit Na-K ATPase. The greater magnitude of the renal response to ANF and the shorter time course of action compared to “natriuretic hormone” further indicate that the two are different substances. Finally, the two differ in their source of origin and affect intrarenal tubular transport of sodium at different sites.

If ANF does indeed play a physiological role in the regulation of sodium excretion, the generation and maintenance of hypertension in some models could involve increased plasma levels not of endogenous Na-K ATPase inhibitor, but instead enhanced release of ANF. The recent findings of Pamnani et al. that increased systemic release of ANF may be associated with reduced atrial tissue storage.

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