Vol. 136, No. 1, 1986 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 14, 1986 Pages 411-416

INCREASED PLASMA IMMUNOREACTIVE ATRIAL NATRIURETIC FACTOR CONCENTRATIONS IN SALT SENSITIVE DAHL RATS

Jolanta Gutkowska, Otto Kuchel[1], Karoly Racz, Nguyen T. Buu, Marc Cantin and Jacques Genest

Laboratory of the Autonomic Nervous System
Clinical Research Institute of Montreal
Université de Montréal and Hôtel-Dieu Hospital
110 Pine Avenue West
Montreal, QUEBEC (H2W 1R7) Canada

Received January 17, 1986

SUMMARY: We measured the immunoreactive atrial natriuretic factor concentrations in plasma and right and left atria of salt-sensitive, salt-resistant Dahl rats and Wistar Kyoto rats, all fed for 5 weeks by 8% salt diet. We found an increase in plasma immunoreactive atrial natriuretic factor ($p < 0.001$) in salt-sensitive Dahl rats which became severely hypertensive in comparison with salt-resistant and Wistar Kyoto rats which remained normotensive on the same diet. There were however, no differences in the immunoreactive atrial natriuretic factor concentrations in the atria between the three groups of rats; all rats tended to have lower concentrations in the left than in the right atrium. The data show the presence of increased circulating atrial natriuretic factor immunoreactivity in hypertensive salt-sensitive Dahl rats which may be due either to the hypertension-induced left atrial distention, to volume expansion or indirectly renal hyposensitivity to the atrial natriuretic factor in these rats.

The recently discovered peptides, isolated from mammalian atria, known under the term of ANF are probably involved in the control of renal water and sodium excretion (1). The role of ANF has also been suggested in the development of different forms of hypertension. With the presently well established structure and increasing recognition of ANF as a circulating hormone involved in the cardiovascular homeostasis, the study of circulating ANF in different models of experimental hypertension can be undertaken. Dahl rats are of particular interest because their hypertension is most clearly related to disturbances in the sodium balance.

[1] To whom correspondence should be addressed.

Abbreviations: ANF: Atrial natriuretic factor, Dahl S: Dahl salt-sensitive rats, R rats: Resistant rats, RIA: Radioimmunoassay, IR-ANF: Immunoreactive atrial natriuretic factor, EDTA: Disodium ethylendiamine tetraacetate, PMSF: Phenylmethyl sulfonyl fluoride, WKY: Wistar Kyoto rats.

0006-291X/86 $1.50

Most recent studies suggest that Dahl S rats have an increased atrial content of ANF but are hyporesponsive to it (2), which may be related to two defects, one involving hyporesponsive kidneys, another decreased release of ANF from atria (3). All these studies were performed by a bioassay technique. The newly developed RIA for ANF (4) allowed us to investigate the role of ANF in the hypertensive Dahl S rats by concomitant measurement of immunoreactive (IR-ANF) atrial and plasma concentrations of ANF. The data, although limited only to the peak state of high salt induced hypertension in S rats indicate a significant elevation of plasma IR-ANF in these rats but no difference in the atrial ANF immunoreactivity.

MATERIALS AND METHODS

Twenty-two male rats of the R strain and 20 of the S strain of Dahl rats from Brookhaven National Laboratory, Upton, N.Y., were used in two independent experiments. Since the appropriateness of the normotensive control groups is often questioned, we added to the first experiment 12 Wistar Kyoto rats of comparable age and exposed them to the same procedures as a control normotensive group of Dahl R rats derived from the Sprague-Dawley strain. The rats were delivered to our animal house a few days before weaning and kept on ordinary chow diet (Na^+, 0.36%; K^+ 1.08%) and water ad libitum for 10 days. After a 10-day-acclimation period, the animals were fed a diet containing 8% NaCl (Ralston Purina Corp., Indiana City, Indiana) for 5 weeks. Food and water were provided ad libitum including the day of urine collections. The rats' weight was taken before and at the end of the experiment and their food intake compared. The systolic blood pressure was measured by a tail cuff method (5). For each rat, 24 hour urinary collections were obtained by housing the rats in individual metabolic cages the day preceeding the end of the experiment. The urine samples were analysed for sodium and potassium content by flame photometry.

At the end of the 5 week high salt diet period, the rats were anesthesized with Pentobarbital (Somnotol 0.4 ml/rat) and 2 ml blood was taken from the abdominal aorta into chilled tubes containing proteases inhibitors to a final concentration: EDTA (1 mg/ml), pepstatin 5 μM and PMSF 10 μM. The plasma was immediately separated and ANF extracted using activated Vycor glass beads as previously described (4). The IR-ANF content in dried extract was measured by radioimmunoassay (6). The hearts from anesthetized rats were quickly removed and washed in saline. The left and right atria were separated and extracts were prepared by homogenizing the tissue in 2 ml of 0.1 M acetic acid containing inhibitors of proteases in the same concentration as in plasma. The homogenates were centrifuged at 30 000 rpm for 20 min at 4°C and kept frozen until the assay. Before the assay, another centrifugation of atrial homogenates in the same conditions was performed and the dilutions of 1:2 000 to 1:8 000 assayed in RIA (6). The data were statistically evaluated by unpaired "t" test and where three groups were involved (Experiment I) also by analysis of variance.

RESULTS

After 5 weeks on high sodium intake all S rats became hypertensive and had significantly higher blood pressure and, despite comparable salt-intake

TABLE 1

Systolic blood pressure, body weight, salt intake and urinary sodium excretion

		S	R	WKY
Blood pressure (mmHg)	Exp I	210 ± 19 *	108 ± 5	118 ± 7
	Exp II	172 ± 6.4 *	111 ± 3.9	-
Body weight (g)	Exp I	293 ± 9	344 ± 8	268 ± 10
	Exp II	296 ± 10	302 ± 8	-
Sodium intake on the day of urine collection (mmol/24 h) †	Exp II	39.1 ± 0.8	41.1 ± 0.7	-
Urinary sodium excretion (mmol/24 h)	Exp I	22.7 ± 1.8 *	29.9 ± 1.4	27.5 ± 1.9
	Exp II	27.9 ± 1.7 *	35.1 ± 1.8	-

NOTE: values are mean ± SE of groups of 11-12 rats. * $p < 0.05$.
†The mean daily sodium-intake on the fifth week on high salt-intake was comparable in Dahl S and R rats (40.1 ± 0.7 vs 40.5 ± 0.7 mmol/24h).

in Dahl S and R rats, lower sodium excretion than R rats and WKY rats (Table 1). In the first experiment (I) the blood pressure was so high that we lost 4 S rats by cerebral hemorrage, compared to the loss of one S rat in the second experiment (II) in which blood pressure was less elevated. WKY rats remained normotensive despite the high salt intake and had Na^+ excretion closer to R rats. In the two experiments we have found, whether evaluated separately or together, higher ($p < 0.001$) mean plasma IR-ANF concentrations in S rats than R and WKY rats (Table 2). In contrast, there were no dif-

TABLE 2

IR-ANF content in plasma and atria

			S	R	WKY
Plasma pg/ml	Exp. I		154 ± 54 * (n = 6)†	52.4 ± 6.8 (n = 11)	79.6 ± 14 (n = 12)
	Exp. II		142 ± 13 * (n = 9)	65.9 ± 6.6 (n = 11)	-
Atria µg/atrium	Exp. I	Left	7.5 ± 1.4	7.9 ± 2	5.6 ± 0.9
		Right	10.5 ± 2.3	13.8 ± 3.7	11.1 ± 2.0
	Exp. II	Left	4.9 ± 1.0	4.4 ± 0.8	-
		Right	6.7 ± 1.8	7.0 ± 1.7	-

* $p < 0.001$ S rats compared to R rats (in experiment I also to WKY rats).
† The same numbers apply also to the atria. Values are: x ± SE.

ferences in the atrial IR-ANF content between S and R rats in both experiments (WKY rats respectively in the first experiment). In both experiments, the IR-ANF content tended to be lower in the left than in the right atrium in Dahl S and R rats as well as in WKY rats as previously observed in Sprague-Dawley and spontaneously hypertensive rats of the Okamato strain (6,7).

DISCUSSION

These data indicate that the high salt-induced hypertension with decreased urinary sodium excretion in S rats is associated, at the height of hypertension, with an increased concentration of IR-ANF in plasma when compared to R rats and WKY rats excreting more sodium (despite a comparable salt intake in R and S rats) and remaining normotensive. The findings that S rats excreted less urinary sodium than R or WKY rats may be due to their lower gastro-intestinal absorption of sodium. Alternatively, the lower sodium excretion despite higher circulating IR-ANF in S rats may also be compatible with the previously postulated renal resistance to ANF in Dahl S rats (2, 3). We did not observe in the present study differences in the atrial content of IR-ANF between the two groups of rats. This is in accordance with a preliminary report (8) that the cardiac levels of atriopeptin are independent of the hypertensive state of the animal. Differences found in this study and those previously reported (2, 3) may be due to differences between the radioimmunoassay and bioassay systems, to the different mode of data expression (total atrial content vs tissue concentrations of ANF) or to different degrees of hypertension or length and degree of salt loading. The present findings of unchanged atrial immunoreactive ANF in S rats do not necessarily contradict previous reports. There may be an increased atrial content of some natriuretic peptide(s) in S rats which are not immunoreactive but have some biological activity. Experiments are now in progress to assess this possibility.

As previously suggested (7), it is the increased left atrial pressure secondary to hypertension which may stimulate ANF release. Whether the increased plasma ANF concentration in S rats is secondary to their hyperten-

sion, volume expansion or to a previously proposed renal hyposensitivity to ANF (2, 3) which may precede the development of hypertension and sodium retention and be age dependent (3), remains to be elucidated. Although we do not have baseline values of IR-ANF prior to salt loading because of technical difficulties of sampling, the comparable concentrations of plasma and atrial IR-ANF following salt loading in the two normotensive strains of rats (the Dahl R rats and WKY rats) suggests that there are no intrinsic on hypertension independent strain differences in the ANF response of normotensive rats to a prolonged high salt intake. This focuses the interest on differences between the Dahl S and R rats; those are hypertensive and the progressive renal lesions of hypertensive S rats (8) makes difficult to judge whether they are hyporesponsive to ANF indeed as they appear to be during normotensive conditions (2, 3).

Further studies will be needed to establish more firmly the conclusion of this study i.e. that plasma ANF determination can better discriminate the ANF status of the animal than ANF determinations in the atria. This would be expected from the fact that ANF is a circulating hormone (10), while its atrial content depends on the delicate balance between ANF synthesis, storage and release. Hypersecretion of ANF can thus be associated as well with an increased, normal or decreased atrial content of ANF dependent on these variables.

ACKNOWLEDGMENTS

This work was supported by grants from the Medical Research Council of Canada to the multidisciplinary group in hypertension, the National Research Council of Canada and the Ministère de la Science et de la Technologie du Québec.

REFERENCES

1. Cantin, M., and Genest, J. (1985) The heart and the atrial natriuretic factor. Endocr. Rev. 6:107-127, 1985.
2. Hirata, Y., Ganguli, M., Tobian, L., and Iwai, J. (1984) Dahl S rats have increased natriuretic factor in atria but are markedly hyporesponsive to it. Hypertension (Suppl I) 6:I-148-155.
3. Snajdar, R.M., and Rapp, J.M. (1985) Atrial natriuretic factor in Dahl rats Atrial content and renal and aortic responses. Hypertension 7:775-782.

4. Horky, K., Gutkowska, J., Garcia, R., Thibault, G., Genest, J. and Cantin M. (1985) Effect of different anesthetics on immunoreactive atrial natriuretic factor concentration in rat plasma. Biochem. Biophys. Res. Comm. 129:651-657.
5. Pfeffer, J., Pfeffer M., and Frohlich, E. (1971) Validity of an indirect tail cuff method for determining systolic arterial pressure in unanesthesized normotensive and spontaneously hypertensive rats. J. Lab. Clin. Med. 78:957-961.
6. Gutkowska, J., Thibault, G., Januszewicz, P., Cantin, M., and Genest, J. (1984) Direct radioimmunoassay of atrial natriuretic factor. Biochem. Biophys. Res. Comm. 122:593-601.
7. Gutkowska, J., Horky, K., Lachance, A., Racz, K., Garcia, R., Thibault, G., Kuchel, O., Genest, J., and Cantin, M. (1986) Atrial natriuretic factor (ANF) in spontaneously hypertensive rats. Hypertension 8:000-000.
8. Schwartz, D., Currie, M.G., and Needleman, P. (1985) Effects of dietary salt on cardiac atriopeptin levels in the Dahl hypertension rat. Fed. Proc. 44:814 (abstract).
9. Rapp, J.P. (1982) Dahl salt-susceptible and salt-resistant rats. Hypertension 4:753-763.
10. Gutkowska, J., Horky, K., Thibault, G., Januszewicz, P., Cantin, M., and Genest J. (1984) Atrial natriuretic factor is a circulating hormone. Biochem. Biophys. Res. Comm. 125:315-323.