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Effect of Chronic Treatment with Deoxycorticosterone Acetate on Content of a Natriuretic Substance in Atria of Rats[1] (42391)

SUSAN J. HATHAWAY,[2] MELVIN J. FREGLY, KAREN M. WILSON, PAULA E. PAPANEK, AND WILLIAM N. HENLEY

Department of Physiology, University of Florida, College of Medicine, Gainesville, Florida 32610

Abstract. Chronic (72 days) administration of deoxycorticosterone acetate (DOCA), with or without saline as the sole drinking fluid, depleted atria of rats of their diuretic and natriuretic activities. Chronic ingestion of saline as the sole drinking fluid did not affect the diuretic, natriuretic, and kaliuretic activities of atria compared with those of rats receiving water to drink. Since systolic blood pressure of the DOCA-treated group did not differ significantly from that of the untreated control group, the decrease in potency of atrial extract from DOCA-treated rats most likely occurred in response to increases in extracellular and vascular volumes. The ability of DOCA to decrease diuretic and natriuretic activities of atria was dose dependent. The decreased activities of the atria of DOCA-treated rats could reflect an increased production and turnover of atrial natriuretic factor. Additional studies revealed an increased diuretic and natriuretic responsiveness of DOCA-treated recipients to atrial extract from untreated rats. Thus, the results of these studies suggest that chronic treatment with DOCA reduced the natriuretic and diuretic potencies of atrial extract and increased renal responsiveness to it.

Extracts prepared from rat atria possess both natriuretic and diuretic activities, and are capable of producing both vasorelaxation of smooth muscle *in vitro* and reducing blood pressure of rats *in vivo* (1–4). The active component of atrial extract (AE) appears to be a heat stable polypeptide(s). Various polypeptide fragments have been isolated from atrial extract, characterized, and synthesized (1, 2). These fragments are believed to be derived from a large (152 amino acid, rat) polypeptide. A portion of this polypeptide (approximately 25 amino acids) appears to possess diuretic, natriuretic, and vasodepressor activities. Although various names have been given to the different fragments, they are usually referred to collectively as atrial natriuretic factor (ANF). Immunocytochemistry has shown the presence of these fragments in granules found within the atria but not in the ventricles (5–8). These granules contain the precursor of ANF.

ANF is postulated to be part of a feedback mechanism designed to maintain a constant extracellular fluid (ECF) volume (1, 2). When ECF volume increases, ANF is secreted to increase urine output and urinary sodium and potassium outputs, thereby returning ECF volume to normal. Thus, ANF may play a role in body fluid homeostasis.

It is unclear at present how ANF produces its renal actions. It has been shown to interfere with the secretion of aldosterone by the adrenal cortex in rats (9, 10). This effect is unlikely to be involved in the acute natriuresis (within 5 min) accompanying a single injection of ANF since an effect on aldosterone secretion is not observed until 30 to 60 min after injection (1–3). Some studies also suggest that diuresis and natriuresis are induced by a direct effect of ANF on renal tubules to inhibit tubuloglomerular feedback (11). Other evidence suggests both an increase and no change in glomerular filtration rate (1), depending upon the way ANF was administered (i.e., infusion vs bolus injection), while still other results suggest that ANF produces its effects primarily as a result of hemodynamic changes independent of tubular transport alterations (12, 13).

The objective of the present studies was to assess the effect of chronic treatment of rats with deoxycorticosterone acetate (DOCA), a

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mineralocorticoid hormone known to induce an expansion of ECF volume (14, 15) on atrial content of ANF.

Methods. *Administration of DOCA.* Female rats of the Blue Spruce Farms (Sprague–Dawley) strain weighing 200–260 g were used. They were kept three per cage in a room maintained at 25°C and illuminated from 0700 to 1900 hr. All rats were provided with Purina Laboratory Chow and either 0.9% NaCl solution or water as drinking fluid, as designated below. DOCA was administered by way of Silastic tubes implanted subcutaneously between the shoulder blades while the rat was anesthetized with ether. Controls were implanted with an empty Silastic tube. Prior to implantation, and after removal at the end of the experiment, the tubes were placed in a vacuum desiccator for 72 hr and weighed on an analytical balance. Daily drug dose was calculated from the weight loss of each tube divided by the number of days the tubes were implanted. Dimethylpolysiloxane (Silastic) tubing has been shown to allow diffusion of certain crystalline steroids into various media at a constant rate over relatively long periods of time (16, 17). In general, the above procedure was followed in the three studies reported here. The variations in this protocol included the length and width of the Silastic tubing implanted (and therefore the dose of DOCA delivered), the duration of implant, and the drinking fluid allotted to the rats (water or 0.9% saline).

Study 1. This study used 24 rats, 12 of which were implanted with tubes 21 mm long (602-235; 1.47 mm i.d., 1.96 mm o.d.). The dose of DOCA delivered by this size of tubing was 66 μg/day. Half of the controls (six) and half of the DOCA-treated rats (six) were allowed 0.9% saline as their drinking fluid. The remaining rats (six DOCA-treated and six control) were given tap water. The duration of treatment was 72 days.

Study 2. Sixty rats, divided into five equal groups, were used in this study. Silastic tubing of four different sizes was implanted into four of the five groups. The dimensions of the tubes and dose of DOCA delivered were the following: 10 mm long (602-235; 1.47 mm i.d., 1.96 mm o.d.; 36 μg/day), 21 mm long (602-285; 1.58 mm i.d., 3.18 mm o.d.; 41 μg/day), 21 mm long (602-265; 1.58 mm i.d., 2.41 mm o.d.; 55 μg/day), and 21 mm long (602-235; 1.47 mm i.d., 1.96 mm o.d.; 66 μg/day). The remaining group was implanted with an empty (21 mm long) tube. The duration of treatment was 35 days during which all rats received Purina Laboratory Chow to eat and tap water to drink.

Study 3. Ten rats were divided equally into two groups (DOCA treated and control). The treatment consisted of weekly injections (12.5 mg/kg, sc) of DOC pivalate for 91 days. Both groups were provided with saline to drink.

Blood pressure. Systolic blood pressures of the rats in Studies 1 and 3 were measured prior to death using the technique described by Fregly (18) and a NarcoBio Instruments Company polygraph to record the pressures.

Preparation of atrial extract. Following decapitation, the atria and apex of the ventricle were quickly excised from the rats in the various groups and placed in ice-cold saline. The tissue was homogenized and then centrifuged at 10,000*g*, 4°C, for 20 min. The supernatant was placed in a boiling water bath for 10 min and then centrifuged at 4000*g*, 4°C, for 15 min. This supernatant was decanted, lyophilized overnight, and stored at −4°C until bioassay was carried out. Reconstitution of the lyophilized atrial extract with 0.9% saline was done on a basis of one atrium/0.1 ml final concentration just prior to bioassay. Ventricular extract (VE) from the same group of rats was reconstituted according to the volume used for the corresponding AE. Protein concentration of the extract was determined by the Coomassie blue method (19).

Bioassay of AE and VE. Male and female Blue Spruce Farms rats (Sprague–Dawley strain, 250–350 g) were used to bioassay the various tissue extracts according to methods previously reported (20). Briefly, rats were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg body wt). Catheters (PE 50) were inserted into the jugular vein for constant infusion of 0.9% saline (1 ml/hr). The bladder was catheterized (PE 50) for urine collection, and the trachea (PE 240) to prevent obstruction of breathing. A minimum of 2 hr was allowed for the animal to recover from the effects of surgery and stabilization of urine output. Body temperature was maintained at 38.0 ± 0.5°C by means of

a warming pad. After three successive stable 15-min urine collections, AE was administered iv. The dose was 1 atrial equivalent (100 μg protein/300 g body wt). The dose of VE was 100 μg protein/300 g body weight. The experiments were performed on pairs of rats. In Study 1 extract from a control group was administered to one rat while extract from a DOCA-treated rat was administered to the other. In Study 2, paired recipient rats received atrial extract from donor rats treated with graded doses of DOCA. In Study 3 the recipient rats, i.e., DOCA-treated and control, received atrial extract from normal rats. Volume of each urine collection was determined gravimetrically. Urinary sodium and potassium concentrations were measured by flame photometer using lithium as the internal standard. Each bioassay rat received only one injection of AE and the corresponding VE. In the first and second studies, AE of DOCA-treated and control rats that had received either saline or water as their sole drinking fluid was administered to normal male or female recipient rats. In the third study, AE, prepared from atria of normal rats, was administered to female recipients that had either received DOC pivalate or served as their controls. Both groups received only saline to drink.

Statistics. Comparison between groups in Studies 1 and 3 was made by means of a paired *t* test in which the differences between the responses of paired DOCA-treated and control rats were used for the analysis. A one-way analysis of variance was used to compare the results among the groups in Study 2 (21). Post hoc comparisons were performed using a Newman–Keuls test. In all cases, significance was set at the 95% confidence limit.

Results. In the first study, chronic treatment with DOCA (66 μg/day) significantly ($P < 0.01$) reduced the diuretic and natriuretic potencies of atrial extract compared with those of atrial extract prepared from controls whether water or saline was given as the sole drinking fluid (Figs. 1A and B present change from baseline values). There were no significant differences between the responses of recipient rats to atrial extracts from the saline- and water-treated control groups.

In the second study, a series of doses of DOCA (0, 36, 41, 55, and 66 μg/day) was administered for 35 days to rats receiving water to drink. Tests of atrial extract from these animals showed a significant dose-response relationship between dose of DOCA administered and the change from baseline of diuretic [$F(4,57) = 3.60$, $P < 0.01$] and natriuretic [$F(4,57) = 3.17$, $P < 0.01$] activities of the extract (Fig. 2). Potencies decreased as dose of DOCA increased until the response was similar to that produced by administration of ventricular extract. The dose-response relationship between dose of DOCA administered and the change from baseline of the kaliuretic activity of the extract did not quite reach the level of significance [$F(4,57) = 2.36$, $P = 0.06$]. Responses to administration of VE in the five groups did not differ significantly one from the other and were therefore combined (Fig. 2). These values for VE were also not significantly different in any case from baseline (i.e., administration of isotonic saline) control values.

In the third study, DOC-treated rats received AE from normal rats. They had both a greater diuretic and natriuretic response than controls (Figs. 3A, B). However, there was no significant difference in potassium output in the DOC-treated group. The protein concentration of atrial extract from the control and DOC-treated groups were 93.5 ± 4.8 (SE) and 82.4 ± 5.6 mg/100 g, respectively. Systolic blood pressures and body weights of treated and control groups in Experiments 1 and 3 also failed to differ significantly from each other (Table I).

Discussion. Present evidence suggests that ANF may be an endogenous hormone. ANF has been shown to be present in granules in the heart (1, 5), and receptors for ANF have been identified in a number of organs, including the kidneys (1, 22). The activity of synthetic ANF also appears to be similar to the native substance (1). The recent ability to measure ANF in the blood supports the hypothesis that ANF is secreted into blood from the heart and is carried to the kidneys where it induces diuresis and natriuresis. However, a physiological role of ANF has yet to be elucidated.

The changes in the natriuretic activity of atrial extract as a result of changes in extracellular fluid volumes suggests that the ulti-

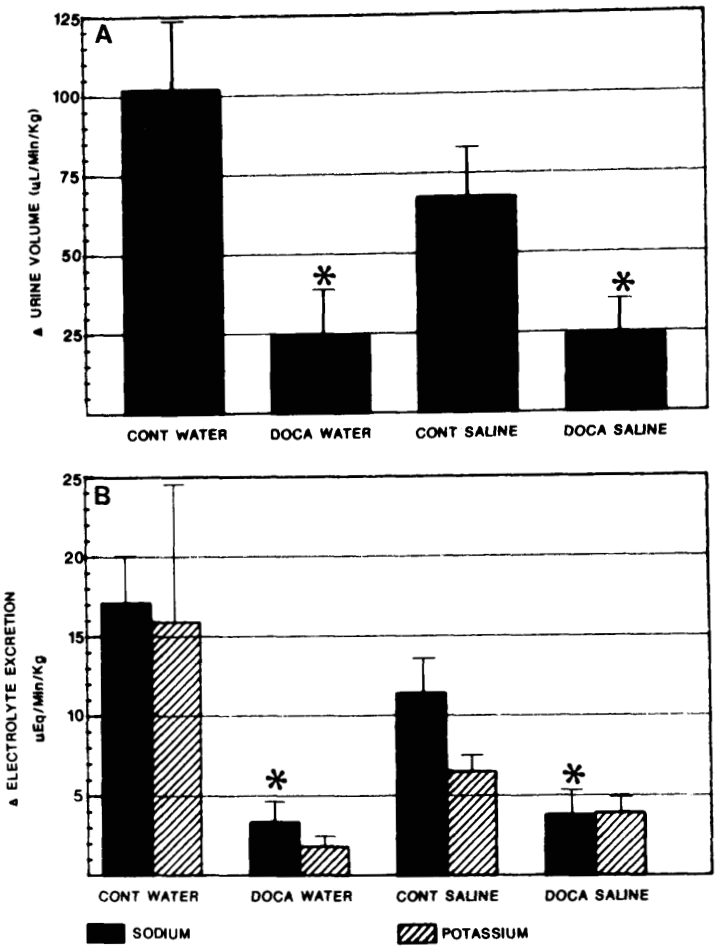

FIG. 1. Effect of administration of extracts of atria from rats treated chronically with either deoxycorticosterone acetate (DOCA, 66 μg/day) and water or DOCA and isotonic saline to drink on change from baseline level in urine flow rate (A) and sodium (black rectangle) and potassium (striped rectangle) excretion rates (B) of untreated recipient rats. One standard error is set off at each mean. $*P < 0.01$.

mate role of ANF may be its involvement in body fluid homeostasis. However, there is at present considerable conflict regarding this potential role for ANF. For example, Johnson (23) reported that atria from water-deprived rats (with a reduced extracellular fluid volume) had an increased natriuretic activity compared with that of controls. In contrast, Thibault *et al.* (24) reported a decreased atrial content of natriuretic activity under similar circumstances. However, the results of the two studies may be difficult to compare since the two groups of investigators used different procedures to extract the natriuretic factor.

When extracellular fluid volume was reduced by chronic dietary sodium deficiency, Pollock and Banks (25) reported that after either 1 or 3 weeks of sodium deficiency, diuretic, natriuretic, and kaliuretic activities of atria were significantly greater than those of the control group which received Purina Laboratory Chow (0.4% sodium). These investigators also gave rats Purina Laboratory Chow and isotonic saline to drink for either 1 or 3 weeks. They reported significant increases in diuretic, natriuretic, and kaliuretic activity of atria from these groups compared to rats given the same diet and water to drink. Similar re-

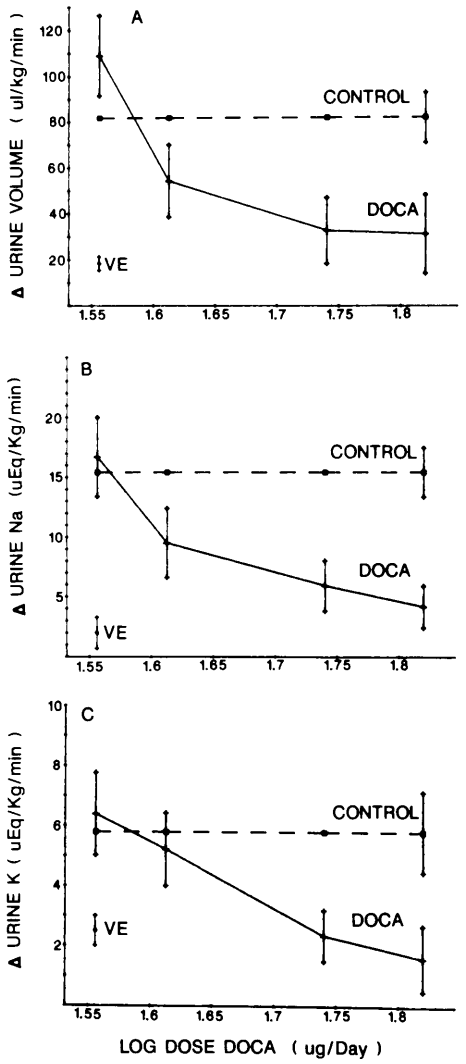

FIG. 2. Effect of administration of extracts of atria from rats treated chronically with graded doses of DOCA on change from baseline (isotonic saline administration) level in urine flow rate (A), urine sodium excretion rate (B), and urine potassium excretion rate (C) of untreated recipient rats. One standard error is set off at each mean. Dashed line represents the response to atrial extract from untreated control rats. VE indicates response to ventricular extract.

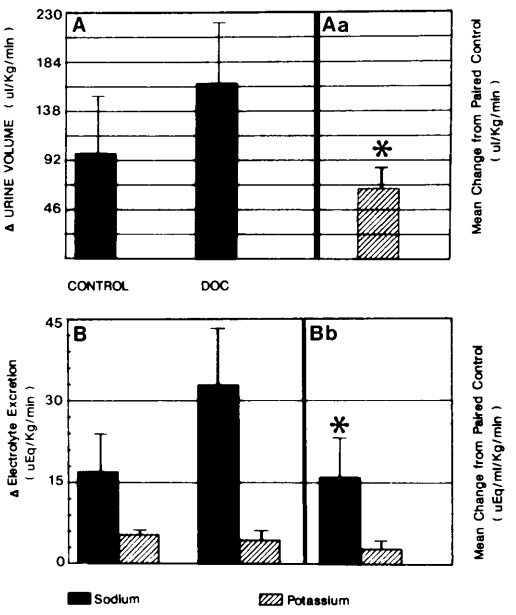

FIG. 3. Effect of administration of extracts of atria from untreated rats on change from baseline level in urine flow rate (A) and urine sodium (black rectangle) and potassium (striped rectangle) excretion rates (B) of recipient rats that had been treated chronically with DOC-trimethyl acetate. Aa and Bb represent the mean change in urine volume and electrolyte excretion (treated minus paired control). One standard error is set off at each mean. $*P < 0.05$.

sults were reported by Hirata *et al.* (26) who gave rats a high salt diet to eat. These results are in disagreement with the findings presented here which show that atria from rats receiving 0.9% saline to drink for 72 days had diuretic and natriuretic activities similar to those of rats given water to drink. All three studies used similar extraction procedures (boiling), but the times during which rats were exposed to salt

TABLE I. EFFECT OF CHRONIC TREATMENT WITH DEOXYCORTICOSTERONE ACETATE (DOCA) AND SALINE ON BODY WEIGHT AND SYSTOLIC BLOOD PRESSURE OF UNANESTHETIZED RATS

Group	*n*	Mean body wt (g)	Systolic blood pressure (mm Hg)
Study 1			
Control + saline	8	296 ± 7[a]	116 ± 4
DOCA + saline (66 μg/day)	8	301 ± 9	117 ± 5
Study 3			
Control + saline	5	308 ± 6	107 ± 4
DOCA + saline (12.5 mg/kg/week)	5	319 ± 6	114 ± 3

[a] One SE of mean.

were different. Hirata *et al.* (26) removed atria after 5 days of a high salt diet. Pollock and Banks (25) found increased activity in the atria after 3 weeks of saline consumption. Thus, the disparity between these studies may be the result of variations in the changes in extracellular and plasma volumes in the different experiments as well as the duration of treatment.

The results of Experiment 2 show that the natriuretic response capable of being produced by the atrial extract of DOCA-treated rats decreased as the dose of DOCA increased until it was similar to that produced by ventricular extract. The graded decrease in levels of natriuretic activity from AE of rats treated with graded doses of DOCA could reflect an inhibitory effect of the mineralocorticoid on production of ANF. The decreases in atrial natriuretic activity may also be the result of continual stimulation of production of ANF by DOCA with a resultant depletion of atrial stores of ANF as fast as it can be produced. The possibility also exists that increased plasma concentration of ANF may actually feed back on the system to inhibit itself. If this were the case, it would be expected that an elevated plasma concentration of ANF would also down-regulate its own receptors. However, DOCA-treated rats responded to AE with an enhanced natriuresis and diuresis. Thus, a supersensitivity to ANF appears to have occurred at the binding sites responsible for the renal effect. Whether this is a reflection of a reduction in the levels of ANF in the blood, or to some aspect of treatment with DOCA, remains for further study. Measurement of immunoassayable ANF under these conditions may provide additional insight.

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1. Needleman P, Adams SP, Cole BR, Currie MG, Geller DM, Michener ML, Saper CB, Schwartz D, Standaert DG. Atriopeptins as cardiac hormones. Hypertension 7:469–482, 1985.
2. Maack T, Camargo MJF, Kleinert HD, Laragh JH, Atlas SA. Atrial natriuretic factor: Structure and functional properties. Kidney Int **27**:607–615, 1985.
3. Grantham JJ, Edwards RM. Natriuretic hormones: At last, bottled in bond? J Lab Clin Med **103**:333–336, 1984.
4. Garcia P, Thibault G, Gutkowska J, Horky K, Hamet P, Cantin M, Genest J. Chronic infusion of low doses of atrial natriuretic factor (ANF Arg 101-Tyr 126) reduces blood pressure in conscious rats without apparent changes in sodium excretion. Proc Soc Exper Biol Med **179**:396–401, 1985.
5. De Bold AJ. Tissue fractionation studies on the relationship between an atrial natriuretic factor and specific atrial granules. Canad J Physiol Pharmacol **60**:324–330, 1982.
6. Cantin M, Gutkowska J, Thibault G, Milne RW, Ledoux S, Minli S, Chapeau C, Garcia R, Hamet P, Genest J. Immunocytochemical localization of atrial natriuretic factor in the heart and salivary glands. Histochemistry **80**:113–127, 1984.
7. Metz J, Mutt V, Forssman WE. Immunohistochemical localization of cardiodilatin in myoendocrine cells of the cardiac atria. Anat Embryol **170**:123–127, 1984.
8. Tang J, Fei H, Xie CW, Suen MZ, Han JS. Characterization and localization of atriopeptin in rat atrium. Peptides **5**:1173–1177, 1984.
9. Atarashi K, Mulrow PJ, Franco-Saenz R, Snajdar R, Rapp J. Inhibition of aldosterone production by an atrial extract. Science **224**:992–994, 1984.
10. Chartier L, Schiffrin E, Thibault G. Effect of atrial natriuretic factor (ANF)-related peptides on aldosterone secretion by adrenal glomerulosa cells: Critical role of the intramolecular disulfide bond. Biochem Biophys Res Commun **122**:171–175, 1984.
11. Briggs JP, Steipe B, Schubert G, Schnermann J. Micropuncture studies of the renal effects of atrial natriuretic substance. Pflugers Arch **395**:271–276, 1982.
12. Borenstein HG, Cupples WA, Sonnenberg H, Veress AT. The effects of natriuretic atrial extract on renal haemodynamics and urinary excretion in anesthetized rats. J Physiol (London) **334**:133–140, 1983.
13. Sonnenberg H, Cupples WA. Intrarenal localization of the natriuretic effect of cardiac atrial extracts. Canad J Physiol Pharmacol **60**:1149–1152, 1982.
14. Villamil MF, Amorena C, Ponce-Hornos J, Muller A, Taquini AC. Role of extracellular volume expansion in the development of DOC-salt hypertension in the rat. Hypertension **4**:620–624, 1982.
15. Braun-Menendez E. The mechanism of hypertension due to desoxycorticosterone. In: Bell ET, ed. Hypertension. Minneapolis, Univ. of Minnesota Press, pp133–146, 1951.
16. Dzuick PJ, Cook B. Passage of steroids through silicone rubber. Endocrinology (Baltimore) **78**:208–211, 1966.
17. Kincl FA, Bonagiano G, Angee I. Sustained release preparations. I. Diffusion of various steroids through polymer membranes. Steroids **11**:673–680, 1968.
18. Fregly MJ. Factors affecting indirect determination of systolic blood pressure of rats. J Lab Clin Med **62**: 223–230, 1963.
19. Bradford MJ. A rapid and sensitive method for the

quantitation of microgram quantities of protein utilizing the principle for protein-dye binding. Anal Chem **72**:248–254, 1975.
20. Hathaway S, Solomon S. The effect of 6-hydroxydopamine on the renal response to atrial natriuretic extract and blood volume expansion. Proc West Pharmacol Soc **27**:89–93, 1984.
21. Snedecor GW, Cochran WG. Statistical Methods. Ames, Iowa State Univ. Press, 5th ed., pp237–328, 1956.
22. Napier MA, Vandlen RL, Albers-Schonberg G, Nutt RF, Brady S, Lyle T, Winquist R, Faison EP, Heinel LA, Blaine EH. Specific membrane receptors for atriopeptin factor in renal and vascular tissues. Proc Natl Acad Sci USA **81**:5946–5950, 1984.
23. Johnson MD. An improved bioassay method for determining natriuretic activity of atrial extracts. Amer J Physiol **248**:F314–F318, 1985.
24. Thibault G, Garcia R, Cantin M, Genest J. Atrial natriuretic factor: Characterization and partial purification. Hypertension **5**:I75–I80, 1983.
25. Pollock DM, Banks RO. Influence of dietary sodium on the natriuretic activity of atrial extracts. Mineral Elect Metab **10**:337–342, 1984.
26. Hirata Y, Tomita M, Yoshimi H, Ikeda M. Specific receptors for atrial natriuretic factor (ANF) in cultured vascular smooth muscle cells of rat aorta. Biochem Biophys Res Commun **125**:562–568, 1984.

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