PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 185, 448–454 (1987)

Role of Atrial Natriuretic Peptide in Mineralocorticoid Escape Phenomenon in Patients with Primary Aldosteronism (42568)

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Abstract. The withdrawal effect of spironolactone treatment on natriuresis was studied in relation to atrial natriuretic peptide (ANP) in five patients with primary aldosteronism due to adenoma. The patients had been treated with spironolactone for 2–3 months before they were admitted. After admission, blood pressure, body weight, and urinary excretion of sodium were measured daily. Venous samples were obtained twice a week for measurements of plasma levels of ANP, plasma renin activity (PRA), and plasma concentrations of aldosterone (PAC), cortisol, and deoxycorticosterone. The study was performed for 7 days during the treatment with spironolactone and for 18 days after stopping the administration. Plasma volume was determined two times, during the control period and on the 13th day after stopping spironolactone. Urinary sodium excretion decreased initially and returned to the control levels successively. Body weight and plasma volume increased, and blood pressure rose steadily. PRA and the plasma concentrations of cortisol and deoxycorticosterone decreased significantly ($P < 0.05$); however, high levels of PAC did not alter significantly. Plasma ANP levels increased significantly ($P < 0.05$) from 26 ± 4 pg/ml during the control period to 195 ± 47 pg/ml on the 13th day after stopping spironolactone. The data of the urinary sodium excretion showed the escape from sodium-retaining effect of aldosterone, and this escape could be explained by the increase in plasma ANP. Furthermore, ANP might contribute to the decrease in cortisol and deoxycorticosterone in plasma because of the direct inhibitory action of ANP on steroidogenesis.

A large body of evidence has indicated that the expansion of body fluid volume is an important factor in the genesis of some forms of hypertension (1). However, the mechanisms of the volume-dependent hypertension induced by an excess of mineralocorticoids are still unclear. In general, the exogenous administration of mineralocorticoids results in transient sodium retention followed by a return of sodium excretion toward a level of sodium intake (2, 3). The term "escape" was introduced into this phenomenon, by which patients with primary aldosteronism do not develop any massive salt retention and edema (3). Humoral factors as well as hemodynamic and physical alterations have been suggested to be involved in this phenomenon (2).

Recently, it is reported (4) that mammalian atria contain peptides with potent diuretic, natriuretic, and vasorelaxing properties. This family of cardiac peptides, collectively called atrial natriuretic peptides, is implicated in the regulation of sodium and volume homeostasis and possibly in the pathogenesis of hypertension (5). Several human atrial natriuretic peptides have been purified, sequenced, and synthesized. Synthetic atrial natriuretic peptide (ANP) causes natriuresis and diuresis and decreases blood pressure when injected into healthy volunteers (6).

We have examined the role of ANP in the escape phenomenon and in the production of steroid hormones during the development of hypertension in patients with primary aldosteronism after cessation of the treatment with spironolactone.

Materials and Methods. Five patients with primary aldosteronism, ranging in age from 24 to 54 years (42 ± 4 years, mean ± SEM), were studied. Informed consent was obtained from each patient prior to the study. The diagnosis was established based on clinical signs and laboratory analyses. All the patients were subsequently operated on and adrenocortical adenoma was histologically demonstrated in

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0037-9727/87 $1.50

each case. The patients had been treated with spironolactone (50–150 mg/day) for 2–3 months before they were admitted. Blood pressure was well controlled and serum potassium was within normal range. After admission, they were on a diet containing 85 meq of sodium and 80 meq of potassium daily. The study was carried out for 7 days during the treatment with spironolactone and for 18 days after withdrawal of the treatment.

Each patient remained in the recumbent position for at least 2 hr on the day when data were collected. Venous samples were obtained twice a week for measurements of plasma ANP levels, plasma renin activity (PRA), and plasma concentrations of aldosterone (PAC), cortisol, and deoxycorticosterone. Serum electrolytes and creatinine were also measured twice a week. Plasma volume was determined by the radioisotope dilution method using ^{125}I-human serum albumin (7) during the control period and on the 13th day after stopping the treatment. Blood pressure in the lying was measured with a sphygmomanometer at 8 AM every day. Daily measurements of body weight and urinary electrolytes were also made. Completeness of 24-hr urine collection was checked by urinary creatinine determinations. Sodium and potassium in serum and urine were measured by flame photometry; calcium was measured by atomic absorption spectrophotometry. Creatinine in serum and urine were determined by the Jaffe reaction (8). PRA, PAC, and plasma concentrations of cortisol and deoxycorticosterone were measured by radioimmunoassay as reported previously (7).

The determination of plasma ANP was performed according to the methods by Naruse *et al.* (9). Blood samples were withdrawn into chilled plastic tubes containing aprotinin (500 kallikrein inactivator units/ml) and 4 m*M* EDTA. Plasma was immediately separated by centrifugation at 4°C and stored at −80°C until further processing. One milliliter of each plasma was acidified by the addition of 1 ml of 4% acetic acid and applied to C18 octadecylsilane cartridges (Sep-PAK C18 cartridge, Waters Associates, Milford, MA) which were pretreated successively with 5 ml of acetonitril and 10 ml of distilled water. The cartridges were then washed with 10 ml of 4% acetic acid, and the samples were eluted with 2 ml of 60% of acetonitril in 0.5% of ammonium acetate. The elutes were evaporated, lyophilized, reconstituted with the assay buffer, and subjected to radioimmunoassay. Synthetic α-human atrial natriuretic peptide (α-hANP) (10) was purchased from Peninsula Laboratories (Belmont, CA). The efficiency of the extraction procedure was estimated by recovery of synthetic α-hANP added to plasma. When 50–500 pg/ml synthetic α-hANP was added, plasma recovery was 81 ± 2% (mean ± SEM, n = 12) after the extraction and radioimmunoassay procedure.

^{125}I-α-hANP and antibody against Atriopeptin I (11) were kindly provided by Mitsubishi Yuka Laboratory of Medical Science. Iodination with ^{125}I was performed according to the chloramine-T method. The antibody was generated in New Zealand white rabbits immunized with Atriopeptin I coupled to bovine thyroglobulin. This antibody shows a complete cross-reaction with α-hANP, but none with angiotensin II, arginine vasopressin, and spironolactone. The assay buffer of radioimmunoassay was 0.1 *M* Tris acetate, pH 7.4, containing 0.1% bovine serum albumin (Fraction 5, Sigma) and 1 m*M* EDTA. The radioimmunoassay incubation mixture consisted of 100 μl of standard α-hANP, 100 μl of antiserum diluted in the assay buffer, 100 μl of ^{125}I-α-hANP (approximately 10,000 cpm), and 200 μl of the assay buffer. The mixture was incubated for 24 hr at 4°C. Separation of free from antibody-bound ^{125}I-peptide was achieved by the polyethylene glycol method. The lowest level of α-hANP that significantly inhibited ^{125}I-α-hANP-binding to the antibody was 7.0 pg/tube, and 50% inhibition of binding was produced by 95 pg/tube. The intra- and interassay coefficients of variation were 7.0 and 9.1% (n = 10), respectively. The results of radioimmunoassay were expressed as picograms immunoreactive α-hANP.

Data are expressed as means ± SEM. Measurements in each experimental day after stopping spironolactone were compared with the mean levels of two determinations on control days during the treatment with spironolactone by analyses of variance with Dunnett's test (12); a significance level of 5% was chosen. Plasma volume during the control period was compared with that on the 13th day by Student's t test for paired observations.

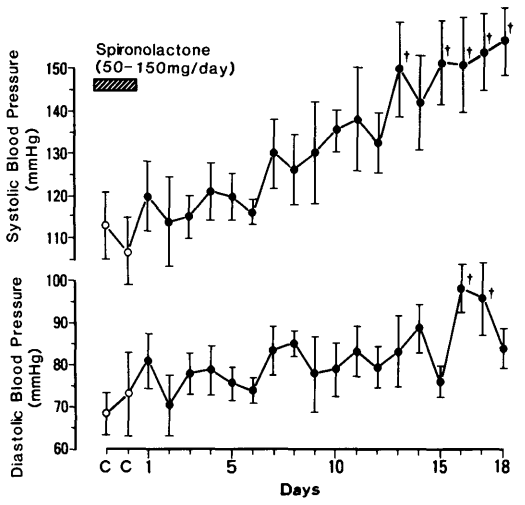

FIG. 1. Changes in systolic and diastolic blood pressure. Data are means ± SEM. Open and closed circles represent the data before and after stopping spironolactone, respectively. C indicates the control days during the treatment with spironolactone. Measurements in each experimental day after stopping spironolactone are compared with the mean levels of two determinations on control days. †$P < 0.05$.

Results. The mean levels of systolic and diastolic blood pressure on the control days averaged 110 ± 8 and 71 ± 4 mm Hg, respectively. Both systolic and diastolic blood pressure increased gradually, and the former rose significantly ($P < 0.05$) to 150 ± 11 mm Hg on the 13th day and the latter to 98 ± 6 mm Hg on the 16th day after stopping spironolactone (Fig. 1).

Average urinary sodium excretion on the control days was 85 ± 6 mmole/day. The excretion decreased initially, reaching the lowest level of 29 ± 7 mmole/day on the 4th day, and returned to the control levels by the 8th day. Urinary potassium excretion increased significantly ($P < 0.05$) from 34 ± 3 mmole/day on the control days to 55 ± 7 mmole/day on the 4th day. Urinary calcium excretion rose significantly ($P < 0.05$) on Days 14 (2.48 ± 0.08 mmole/day) and 15 (2.63 ± 0.10 mmole/day) as compared to the control levels (1.25 ± 0.08 mmole/day; Fig. 2).

High levels of PAC did not alter significantly with withdrawal of spironolactone, while PRA fell markedly from 2.1 ± 0.6 ng/ml/hr on the control days to 0.2 ± 0.1 ng/ml/hr on Day 8. The control levels of plasma ANP averaged 26 ± 4 pg/ml. After stopping the treatment, the concentration of plasma ANP increased significantly ($P < 0.05$) to 145 ± 43 pg/ml on the 8th day when the escape appeared, and reached a peak level of 195 ± 47 pg/ml on the 13th day (Fig. 3). Fig. 4 shows the temporal relationships among plasma ANP levels, PRA, and urinary sodium excretion in each patient, and Table I presents the individual data for PAC. The values for plasma cortisol on the control days (12.8 ± 1.1 μg/dl) decreased significantly ($P < 0.05$) to 8.2 ± 2.8 μg/dl on Day 8. A similar pattern was observed in plasma deoxycorticosterone; the value decreased significantly ($P < 0.05$) from 396 ± 106 pg/ml on the control days to 173 ± 27 pg/ml on Day 8 (Fig. 5).

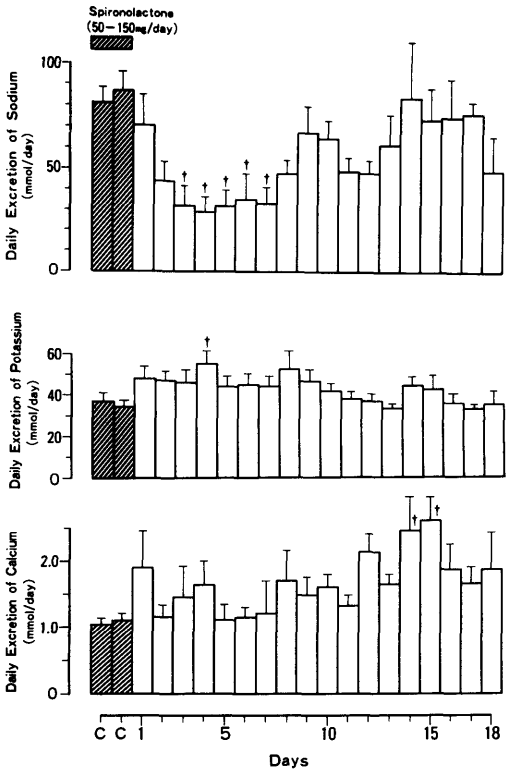

FIG. 2. Changes in daily excretion of sodium, potassium, and calcium. Data are means ± SEM. Shaded and open bars represent the data before and after stopping spironolactone, respectively. C indicates the control days during the treatment with spironolactone. Measurements in each experimental day after stopping spironolactone are compared with the mean levels of two determinations on control days. †$P < 0.05$.

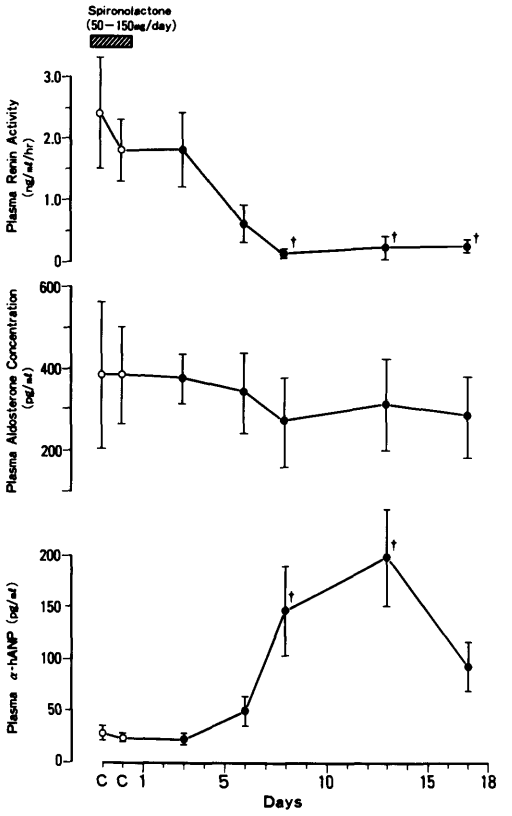

FIG. 3. Changes in PRA, PAC, and plasma levels of immunoreactive α-hANP. Open and closed circles represent the data before and after stopping spironolactone, respectively. C indicates the control days during the treatment with spironolactone. Measurements in each experimental day after stopping spironolactone are compared with the mean levels of two determinations on control days. †$P < 0.05$.

Serum potassium decreased significantly ($P < 0.05$) on the 13th day without significant changes of serum sodium. Serum calcium decreased slightly but the changes were not significant. Creatinine clearance rose significantly ($P < 0.05$) on the 13th day. Both plasma volume and body weight increased significantly ($P < 0.05$). Weight gain on the final day of the study averaged 1.6 ± 0.2 kg (Table II).

Discussion. After stopping the spironolactone treatment, the patients in the present study showed a decrease in urinary sodium excretion, suggesting a sodium retention induced by an excessive amount of aldosterone. However, the excretion returned to the control levels successively despite the high levels of PAC. Body weight and plasma volume increased, whereas edema was not observed in each patient. These observations indicate that the escape from sodium-retaining effect by the excess of aldosterone occurred in the patients. Wenting *et al.* (13) also observed an escape phenomenon similar to that in the present study in patients with primary aldosteronism after withdrawal of spironolactone.

Plasma ANP concentrations increased markedly in accordance with the onset of natriuresis, reaching levels of 145 ± 43 and 195 ± 47 pg/ml on Days 8 and 13, respectively. These values seem consistent with those of the plasma ANP levels to cause natriuresis when synthetic ANP is infused into healthy volunteers (14, 15). Because the release of this peptide into circulation is elicited by volume expansion and atrial distension (16), the sodium retention and the expanded volume by the excessive amount of aldosterone may increase ANP in plasma and result in natriuresis. The increased levels of plasma ANP during the escape phenomenon has also been reported by the study of normal human subjects treated with fludrocortisone (17, 18).

The marked suppression in PRA in the present study may be attributed in part to the inhibitory action of ANP on renin secretion (19) in addition to the inhibition by the expanded body fluid volume. A decrease in PRA and angiotensin II levels observed after min-

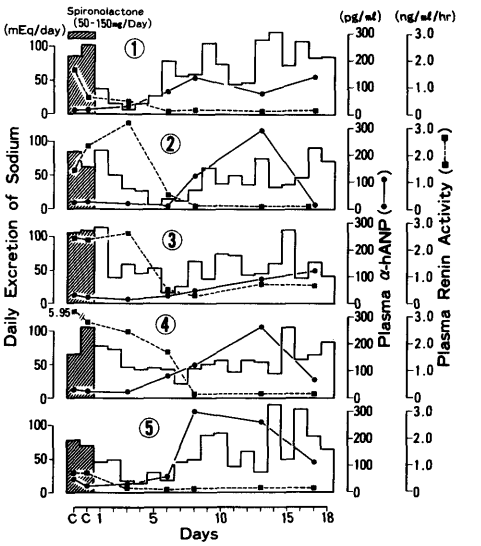

FIG. 4. Plasma ANP levels, PRA, and urinary sodium excretion in five patients.

TABLE I. PLASMA ALDOSTERONE CONCENTRATION IN FIVE PATIENTS (pg/ml)

Patients	Control days		Experimental days after stopping spironolactone				
			3	6	8	13	17
1	1096	852	584	702	701	729	594
2	354	332	328	361	213	195	168
3	151	236	350	189	164	142	79
4	162	170	227	227	162	152	165
5	174	345	383	224	108	332	384

eralocorticoid administration has been suggested to be causally related to the escape phenomenon, because angiotensin II possesses a sodium-retaining action independent of its effect on aldosterone secretion (2). Therefore, ANP could contribute to the escape phenomenon by suppressing renin release and angiotensin II formation. Glomurular filtration rate (GFR) may also play an important role in the escape mechanism. Several investigators have observed an increase in GFR after mineralocorticoid administration when the escape is brought about (20, 21). In the present study, creatinine clearance increased gradually in parallel with plasma ANP. Since the marked increase in GFR following the infusion of ANP has been reported (22, 23), the changes in the creatinine clearance might be mediated through the rise in ANP. Furthermore, an increase in blood pressure, or more importantly renal perfusion pressure, has been shown to play an important role in the escape phenomenon (21). The elevated blood pressure in the present study may have contributed to the escape phenomenon. Several other mechanisms, i.e., renal adrenergic system, kallikrein-kinin, and prostaglandins, have been proposed as causative factors for the escape phenomenon (2). The development of a specific ANP antagonist will determine relative and quantitative importance of ANP in this phenomenon.

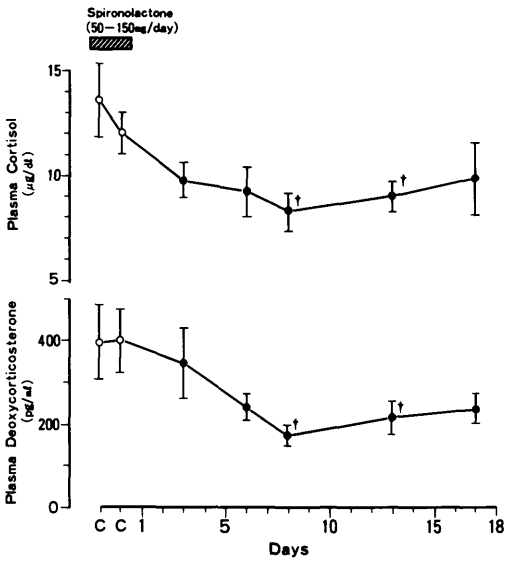

FIG. 5. Changes in plasma levels of cortisol and deoxycorticosterone. Open and closed circles represent the data before and after stopping spironolactone, respectively. C indicates the control days during the treatment with spironolactone. Measurements in each experimental day after stopping spironolactone are compared with the mean levels of two determinations on control days. †$P < 0.05$.

After stopping the treatment, we observed an increase in urinary calcium, and the appearance of hypercalciuria coincided with the highest level of plasma ANP. Serum calcium decreased slightly. These results are in good agreement with the reports (24, 25) that prolonged administration of mineralocorticoids resulted in an increase in urinary calcium associated with the escape phenomenon. Since the urinary output of calcium is raised by ANP administration (6), the hypercalciuria associated with the escape in the present study could be attributable to the alterations in plasma ANP.

The distinct inhibitory effect of ANP infusion on basal plasma levels of aldosterone and cortisol has been reported in normal humans (14). ANP inhibits steroidogenesis in normal adrenal cells *in vitro* at an early step before mitochondrial metabolism of cholesterol and its side-chain cleavage (26). Some investigators (27, 28) studied the effect of ANP on aldosteronogenesis in adrenal adenoma cells *in vitro* from patients with primary aldosteronism.

TABLE II. SERUM ELECTROLYTES, CREATININE CLEARANCE, PLASMA VOLUME, AND BODY WEIGHT BEFORE AND AFTER THE WITHDRAWAL OF THE SPIRONOLACTONE TREATMENT

	Control days		Experimental days after stopping spironolactone				
			3	6	8	13	17
Serum sodium (mmol/l)	140 ± 2	140 ± 2	140 ± 1	141 ± 1	141 ± 1	144 ± 1	142 ± 1
Potassium (mmol/l)	4.3 ± 0.2	4.4 ± 0.1	4.2 ± 0.2	3.8 ± 0.3	3.6 ± 0.3	3.3 ± 0.3†	3.3 ± 0.3†
Calcium (mmol/l)	2.40 ± 0.04	2.37 ± 0.05	2.33 ± 0.05	2.29 ± 0.06	2.24 ± 0.05	2.24 ± 0.06	2.25 ± 0.07
Creatinine clearance (ml/min)	66 ± 5	63 ± 6	60 ± 7	71 ± 9	69 ± 8	94 ± 9†	77 ± 7
Plasma volume (ml/kg)	43 ± 4					49 ± 4†	
Body weight (kg)	53.3 ± 3.9	53.6 ± 3.8	53.5 ± 4.0	54.1 ± 3.8	54.5 ± 3.8	55.1 ± 3.8	55.0 ± 3.8†

Note. Values are means ± SEM. Measurements in each experimental day after stopping spironolactone are compared with the mean levels of two determinations on control days during the treatment with spironolactone.
† $P < 0.05$.

They concluded that ANP lacked an inhibitory effect on aldosteronogenesis in adrenal adenoma cells because of the defect in ANP-specific receptor sites in these cells. These reports support the present findings showing the decrease in plasma cortisol and deoxycorticosterone with no significant alterations in PAC.

The patients in the present study revealed typical clinical features of primary aldosteronism after withdrawal of spironolactone. Blood pressure rose steadily, and serum potassium and PRA fell markedly. The mechanisms of the development of hypertension in mineralocorticoid excess syndrome have not been fully understood; the role of ANP remains to be clarified. The concomitant increase in plasma ANP with the rise in blood pressure suggests that ANP may limit the development of hypertension in these patients; more severe and earlier elevation in blood pressure would have occurred in the absence of ANP, because ANP has been reported to have vasorelaxing activity and to lower blood pressure when infused into normal volunteers (5).

In conclusion, the present study suggests that circulating ANP be related to the escape phenomenon, and that ANP might contribute to steroidogenesis and alterations in renal function associated with natriuresis in patients with primary aldosteronism.

The authors are very grateful to Miss Sayuri Iriya, Miss Akemi Yoguchi, and Mr. Yukihito Fukumura for their skillful technical assistance.

1. Guyton AC, Coleman TG, Cowley AW, Scheel KW, Manning RD, Norman RA. Arterial pressure regulation. In: Laragh JH, ed. Hypertension Manual. New York: Dun-Donnelley, p111-134, 1974.
2. Knox FG, Burnett JC, Jr., Kohan DE, Spielman WS, Strand JC. Escape from the sodium-retaining effects of mineralocorticoids. Kidney Int **17**:263-276, 1980.
3. Schrier RW, Leaf A. Normal physiology of sodium, chloride, and potassium excretion: Effect of hormones on water, sodium, chloride, and potassium metabolism. In: Williams RH, ed. Textbook of Endocrinology. Philadelphia: W B Saunders, p1039-1044, 1981.
4. Sagnella GA, MacGregor GA. Cardiac peptides and the control of sodium excretion. Nature **309**:666-667, 1984.
5. Needleman P, Greenwald JE. Atriopeptin: A cardiac hormone intimately involved in fluid, electrolyte, and blood pressure homeostasis. N Engl J Med **314**:828-834, 1986.
6. Richards AM, Nicholls MG, Ikram H, Webster MW, Yandle TG, Espiner EA. Renal, haemodynamic, and hormonal effects of human alpha atrial natriuretic peptides in healthy volunteers. Lancet **1**:545-548, 1985.
7. Ichikawa S, Tajima Y, Sakamaki T, Matsuo H, Kogure M, Hirano Y, Yagi S, Murata K. Effect of spironolactone on fluid volumes and adrenal steroids in primary aldosteronism. Japan Circ J **48**:1184-1196, 1984.

8. Bonsnes RW, Taussky HH. On the colorimetric determination of creatinine by the Jaffe reaction. J Biol Chem **158**:581–591, 1945.
9. Naruse M, Naruse K, Obana K, Kurimoto F, Sakurai H, Honda T, Higashida T, Demura H, Inagami T, Shizume K. Immunoreactive α-human atrial natriuretic polypeptide in human plasma. Peptides **7**:141–145, 1986.
10. Kangawa K, Matsuo H. Purification and complete amino acid sequence of α-human atrial natriuretic polypeptide (α-hANP). Biochem Biophys Res Commun **118**:131–139, 1984.
11. Currie MG, Geller DM, Cole BR, Siegel NR, Fox KF, Adams SP, Eubanks SR, Gallupi GR, Needleman P. Purification and sequence analysis of bioactive atrial peptides (Atriopeptins). Science **223**:67–69, 1984.
12. Dunnett CW. New tables for multiple comparisons with a control. Biometrics **20**:482–491, 1964.
13. Wenting GJ, Veld AJ, Verhoeven RP, Derkx FH, Schalekamp MA. Volume–pressure relationships during development of mineralocorticoid hypertension in man. Circ Res **40**(suppl 1):163–170, 1977.
14. Weidmann P, Hellmueller B, Uehlinger DE, Lang RE, Gnaedinger MP, Hasler L, Shaw S, Bachmann C. Plasma levels and cardiovascular, endocrine, and excretory effects of atrial natriuretic peptide during different sodium intakes in man. J Clin Endocrinol Metab **62**:1027–1036, 1986.
15. Tikkanen I, Fyhrquist F, Metsärinne K, Leidenius R. Plasma atrial natriuretic peptide in cardiac disease and during infusion in healthy volunteers. Lancet **2**:66–69, 1985.
16. Lang RE, Thölken H, Ganten D, Luft FC, Ruskoaho H, Unger Th. Atrial natriuretic factor—A circulating hormone stimulated by volume loading. Nature **314**: 264–266, 1985.
17. Wambach G, Götz S, Kaufmann W. Plasma levels of atrial natriuretic peptide (ANP) during mineralocorticoid escape in normal man. Klin Wochenschr **64**(Suppl 6):53–57, 1986.
18. Zimmerman RS, Edwards BS, Schwab TR, Heublein DM, Burnett JC, Jr. Atrial natriuretic peptide during mineralocorticoid escape in the human. J Clin Endocrinol Metab **64**:624–627, 1987.
19. Villarreal D, Freeman RH, Davis JO, Verburg KM, Vari RC. Renal mechanisms for suppression of renin secretion by atrial natriuretic factor. Hypertension **8**(Suppl 2):28–35, 1986.
20. Biglieri EG and Forsham PH. Studies on the expanded extracellular fluid and the responses to various stimuli in primary aldosteronism. Amer J Med **30**:564–576, 1961.
21. Hall JE, Granger JP, Smith MJ, Jr., Premen AJ. Role of renal hemodynamics and arterial pressure in aldosterone "escape". Hypertension **6**(Suppl 1):183–192, 1984.
22. Burnett JC, Jr., Opgenorth TJ, Granger JP. The renal action of atrial natriuretic peptide during control of glomerular filtration. Kidney Int **30**:16–19, 1986.
23. Weidmann P, Hasler L, Gnädinger MP, Lang RE, Uehlinger DE. Blood levels and renal effects of atrial natriuretic peptide in normal man. J Clin Invest **77**: 734–742, 1986.
24. Suki WN, Schwettmann RS, Rector FC, Jr., Seldin DW. Effect of chronic mineralocorticoid administration on calcium excretion in the rat. Am J Physiol **215**:71–74, 1968.
25. Massry SG, Coburn JW, Chapman LW, Kleeman CR. The effect of long-term desoxycorticosterone acetate administration on the renal excretion of calcium and magnesium. J Lab Clin Med **71**:212–219, 1968.
26. Goodfriend TL, Elliott ME, Atlas SA. Actions of synthetic atrial natriuretic factor on bovine adrenal glomerulosa. Life Sci **35**:1675–1682, 1984.
27. Hirata Y, Tomita M, Yoshimi H, Kuramochi M, Ito K, Ikeda M. Effect of synthetic human atrial natriuretic peptide on aldosterone secretion by dispersed aldosterone-producing adenoma cells in vitro. J Clin Endocrinol Metab **61**:677–680, 1985.
28. Higuchi K, Nawata H, Kato K, Ibayashi H, Matsuo H. Lack of inhibitory effect of α-human atrial natriuretic polypeptide on aldosteronogenesis in aldosterone-producing adenoma. J Clin Endocrinol Metab **63**:192–196, 1986.

Received December 2, 1986. P.S.E.B.M. 1987, Vol. 185.
Accepted April 23, 1987.