Kaliuretic peptide and long acting natriuretic peptide as well as atrial natriuretic factor inhibit aldosterone secretion

D L Vesely, S Chiou, M A Douglass, M T McCormick, G Rodriguez-Paz and D D Schocken

Departments of Internal Medicine, Physiology and Biophysics, University of South Florida for Health Sciences and James A Haley Veterans Hospital, Tampa, Florida 33612, USA

(Requests for offprints should be addressed to D L Vesely, James A Haley Veterans Hospital – 151, 13000 Bruce B Downs Blvd, Tampa, Florida 33612, USA)

Abstract

The present investigation was designed to determine whether atrial natriuretic peptides consisting of amino acids 1–30 (long acting natriuretic peptide), 31–67 (vessel dilator) and 79–98 (kaliuretic peptide) as well as 99–126 (atrial natriuretic factor (ANF)) of the 126 amino acid ANF prohormone inhibit aldosterone secretion. Thirty healthy human subjects were studied following infusion of 100 ng/kg body weight/min for 60 min of each of the respective peptides. Kaliuretic peptide decreased plasma aldosterone concentration by the greatest amount (6-fold) and plasma aldosterone was still significantly decreased (P<0.001) three hours after stopping the infusion. In contrast, within 30 min of cessation of the ANF infusion, plasma aldosterone levels had returned to pre-infusion values. Long acting natriuretic peptide also significantly (P<0.01) decreased plasma aldosterone levels which remained significantly (P<0.001) decreased 3 h after cessation of infusion. Vessel dilator did not decrease plasma aldosterone levels. Kaliuretic peptide, ANF and long acting natriuretic peptide also decreased (P<0.01) urinary aldosterone concentrations. None of these peptides changed the plasma potassium concentration. We conclude that two new peptide hormones (long acting natriuretic peptide and kaliuretic peptide) inhibit aldosterone secretion. The length of time that aldosterone secretion is inhibited following kaliuretic peptide and long acting natriuretic peptide infusion is significantly longer (P<0.001) than following ANF infusion.

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Introduction

The most important regulators of aldosterone secretion are the renin-angiotensin system and potassium (Quinn & Williams 1988). Atrial natriuretic factor has been found to be a potent in vivo and in vitro inhibitor of aldosterone secretion via a direct effect on the adrenal (Atarashi et al. 1984, Burnett et al. 1984, Chartier et al. 1984, Goodfrield et al. 1984, Kudo & Baird 1984, Campbell et al. 1985, Espiner et al. 1985, Hirata et al. 1985, Anderson et al. 1986, Higuchi et al. 1986, Aguilar 1987, Denker et al. 1990, Clark et al. 1992) and indirectly through inhibition of renin release (Burnett et al. 1984, Maack et al. 1984, Kurtz et al. 1986, Vari et al. 1986). Atrial natriuretic factor also enhances potassium excretion in both animals (Martin et al. 1990) and humans (Vesely et al. 1994b) with the resultant change in the circulating potassium concentration possibly also contributing to the decreased aldosterone secretion that has been observed.

Atrial natriuretic factor (ANF) consists of amino acids 99–126 of the atrial natriuretic factor prohormone. At least three other peptide hormones are derived from this same prohormone (Martin et al. 1990, Gower et al. 1994, Vesely et al. 1994a,b). These peptides, consisting of amino acids 1–30 (pro ANF 1–30; long acting natriuretic peptide), 31–67 (pro ANF 31–67, vessel dilator) and 79–98 (pro ANF 79–98, kaliuretic peptide) of the 126 amino acid atrial natriuretic factor prohormone, have potent diuretic, natriuretic and/or kaliuretic properties in animals (Martin et al. 1990) and humans (Vesely et al. 1994b). Of the four peptide hormones derived from the ANF prohormone, kaliuretic peptide has the most potent effect on potassium metabolism in both humans (Vesely et al. 1994b) and animals (Martin et al. 1990). Whether kaliuretic peptide, long acting natriuretic peptide, and/or vessel dilator may inhibit aldosterone and/or renin release in vivo has never been investigated. The present investigation was designed to determine if any of these peptides have direct effects on aldosterone secretion.

Materials and Methods

Healthy volunteers

Thirty healthy subjects (15 men and 15 women; aged 20–58 years; average, 32 years; all normotensive with...
blood pressures <125/80 mm Hg) were studied. These subjects had heart rates ranging from 56 to 80 beats per minute, with respiration rates between 12 and 14 per minute. The volunteers were divided into five similar groups, based upon age, sex, weight, blood pressure and heart rate. The characteristics of each individual in this investigation have been published recently (Vesely et al. 1994b) in an investigation of potassium and sodium excretion following the administration of each of the atrial peptides. Evaluation of plasma and urine aldosterone responses to infusion of each of the atrial peptides in these same subjects was used in the present investigation to help determine the mechanism of the changes in sodium and potassium excretion found previously. None of the volunteers had any known disease and, importantly, none of the subjects had any abnormality of sodium or water metabolism. None of the volunteers was taking any medication. Informed consent was obtained from each of the volunteers after the nature and possible consequences of the studies were fully explained. This study was approved by the Institutional Review Board of the University of South Florida Health Sciences Center and the Research Committee of the James A Haley Veterans Hospital. This study was also approved by the United States Food and Drug Administration (FDA IND No. 32 119).

**Experimental protocol**

The experimental protocol consisted of a 60-min baseline period preceding any infusion. A total volume of 20 ml normal saline (0.9% sodium chloride, with or without peptides) was infused by a constant-rate infusion pump over a 60-min time period. Blood and urine samples were obtained every 20 min during the infusion and at 30-min time intervals during the 1-h baseline and 3-h post-infusion time periods. The control subjects received vehicle only, but otherwise adhered to an identical protocol of a 1-h equilibration period followed by a 1-h infusion period with a 3-h recovery period of evaluation. One hundred nanograms per kilogram body weight per minute was chosen for the infusion dosage of the atrial natriuretic peptides because the rate of release of the N-terminal ANF prohormone peptides from the atrium of the heart with physiological stimuli is 138–292 ng/kg body wt per min, whereas the release rate of ANF from the atrium is 76 ng/kg body wt per min (Ackerman et al. 1992). Molar equivalents of 100 ng/kg body wt dose are 32, 29, 26 and 46 pmol/kg body wt for ANF, proANFs 1–30, 31–67 and 79–98 respectively. Thus the concentrations used in this investigation are in the physiological range based on the release rates for the N-terminal ANF prohormone peptides and slightly above the physiological range for ANF.

Each of the subjects ingested their usual diet until the evening before the study. All subjects were studied in the morning after an overnight fast, beginning their baseline period at 0800 h. Each volunteer was studied in the seated position. In order to maintain a similar plasma volume throughout the study, after completion of the 60-min baseline period orange juice (Na⁺=0.001 mol/l; K⁺=0.046 mol/l) was given orally in ml for each ml of urine output at the above time periods. Each volunteer received only one peptide infusion.

**Purity of atrial natriuretic peptides**

The human forms of proANFs 1–30, 31–67, 79–98 and ANF (i.e. proANF 99–126) were synthesized by Peninsulal Laboratories, Inc. (Belmont, CA, USA). Before their use in these studies, samples of these commercially synthesized peptides were subjected to high pressure liquid chromatography to determine purity using a Novapak C₁₈ (5 µm) cartridge column, as described previously (Winters et al. 1989). After determining that the peptides were pure, they were dissolved in 0.9% saline solution in the hospital pharmacy, where pyrogen and sterility testing were performed before dispensing the 100 ng/kg body wt concentrations of each peptide into two 10-ml syringes. Each 10-ml syringe was infused over a 30-min time period. After completing the experiments, the syringes and the infusion catheters were examined by radioimmunoassays to determine the amount of the peptides that had remained within the syringes or tubing. Approximately 5% of each peptide remained on the walls of the syringes and tubing after completion of infusion.

**Aldosterone radioimmunoassay**

Aldosterone was measured in the urine with an ethylacetate extraction procedure and in unextracted plasma with an aldosterone radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, CA, USA). This radioimmunoassay utilizes 7 µCi ¹₂⁵I-aldosterone/200 pre-antibody-coated polypropylene 12 × 75 mm tubes. The assay can detect as little as 16 pg/ml (1.6 ng/dl) and has a very low cross-reactivity with other compounds that might be found in the respective samples, e.g. androsterone 0·0005%, cortisol 0·0003%, 11-deoxycortisol 0·0004%, dehydroepiandrosterone (DHEA) 0·0005% and progesterone 0·007%. There was no detectable cross-reactivity with androstendione, cortisol, oestradiol, oestrone, fluocortisone, pregnenolone, 17α-hydroxyprogesterone or testosterone. The cross-reactivity with aldosterone was 100% while the cross-reactivity with the synthetic mineralocorticoid Spirnolactone was 0·06% as determined by Diagnostic Products Corporation. The extraction procedure for measurement of aldosterone in the urine was as follows: 25 µl 3·2 M HCl was added to each 250 µl urine which was incubated in the dark for 24 h at room temperature for hydrolysis. Then 2·5 ml 100% ethylacete was added to each tube and mixed by
gentle inversion for 60 min with a mechanical rocker (Tek-Pro Rocker, American, Dade, Miami, FL, USA), centrifuged at 1500 g for 15 min, and taken to dryness with low flow nitrogen. The samples were then ready for radioimmunoassay. The intra-assay coefficient of variation was 5-5% while the interassay coefficient of variation was 9-4%. The normal range for aldosterone with this assay in human subjects is 4-31 ng/dl for serum or plasma samples and 6-25 μg/day in the urine.

Plasma potassium

Plasma potassium concentrations were measured by flame photometry (Instrumentation Laboratory 943, Lexington, MA, USA) as described previously (Vesely et al. 1994b).

Statistical analysis

The data obtained in this investigation are presented as the mean and s.d.. Measurements obtained in the same subject over time were evaluated by repeated measures of analysis of variance (ANOVA). Duncan’s multiple range test (MRT) was used after ANOVA to evaluate which means were significantly different from baseline and from each other. To be statistically significant, we required a P value to be less than 0.05 (95% confidence limits).

Results

Infusion of atrial natriuretic factor (ANF) significantly (P<0.01) decreased the aldosterone concentration in plasma during the 60 min infusion (Fig. 1). Within 30 min of cessation of the ANF infusion, the concentration of aldosterone had returned to its pre-infusion values (Fig. 1).

In the control subjects who received vehicle only, plasma aldosterone concentration increased during the infusion period, remained elevated at 30 min, and returned to its pre-infusion values by 60 min post-infusion (Fig. 1). Kaliuretic peptide caused a 6-fold decrease in plasma aldosterone concentration (the most of any of the atrial peptides) within the first 20 min of infusion and the concentration remained significantly decreased (P<0.001) three hours after cessation of the kaliuretic peptide infusion (Fig. 1). Long acting natriuretic peptide decreased plasma aldosterone during its infusion and levels remained...
Atrial natriuretic factor has previously been shown to inhibit aldosterone secretion from cultured adrenal cells (Chartier et al. 1984, Goodfrield et al. 1984, Kudó & Baird 1984, Campbell et al. 1985, Higuchi et al. 1986, Aguilar 1987, Denker et al. 1990) and to inhibit secretion of aldosterone in vivo (Espiner et al. 1985, Anderson et al. 1986, Takagi et al. 1986, Vari et al. 1986, Clark et al. 1992). The inhibitory effect of ANF on aldosterone activity is thought to be a direct effect on steroidogenesis inhibiting both aldosterone and 19-hydroxy-androstenedione (an aldosterone amplifier) secretion by adrenal cells (Higuchi et al. 1989). Potassium-stimulated aldosterone secretion is also markedly inhibited by atrial natriuretic factor (Takagi et al. 1986, Clark et al. 1992). The present investigation confirms that ANF inhibits aldosterone secretion in humans and that this effect is due to an effect on release rather than on the metabolism of aldosterone since the concentration of aldosterone following ANF administration decreased in both the plasma and the urine when measured simultaneously and in a time-wise fashion. If the decreased plasma aldosterone concentration was due to increased metabolism its concentration in the urine samples would have been increasing rather than decreasing.

With respect to the metabolism of aldosterone, the liver is the major organ for inactivating steroids via the enzymatic reduction of the 4–5 double bond in the ‘ring A’ of steroids to form a dihydrosteroid derivative, which is quickly converted to a tetrahydro derivative by the enzymatic reduction of the 3-oxo group to a 3-hydro group. Tetrahydroaldosterone formed in this manner is then readily conjugated with glucuronic acid forming a water soluble product that is rapidly excreted by the kidneys. Aldosterone is also very susceptible to conjugation with glucuronic acid at its 18-oxo position. This product is formed in both the liver and the kidney. It is very water soluble and readily excreted into urine. Thus, whether aldosterone is metabolized within the liver or the kidney, this metabolism is reflected in the measured urine concentration of aldosterone. The present investigation demonstrates that even at the first time point (20 min) after infusing the respective atrial peptides, which decreased plasma aldosterone concentrations, the urine concentration of aldosterone had simultaneously decreased in a proportional manner. These findings indicate that when the liver and kidney have a decreased amount of aldosterone presented to them via the circulation, they rapidly adjust to metabolize a lesser amount of aldosterone than they had been metabolizing prior to the decrease in the plasma concentration of aldosterone. It is important to note in this regard that the urine concentrations of aldosterone measured in the investigation are reflecting the metabolism of aldosterone by both the liver and the kidney.

Kaliuretic peptide was found to be an even more potent inhibitor than ANF of aldosterone secretion in healthy humans when both were directly compared at identical concentrations. In these same subjects, kaliuretic peptide has stronger potassium excreting properties than ANF (Vesely et al. 1994b). These findings (Vesely et al. 1994b) suggest that the inhibitory effects of kaliuretic peptide (and ANF) on aldosterone secretion may involve modification of potassium metabolism as well as a direct effect. In the present investigation, however, plasma potassium did not change following administration of any of the atrial peptides suggesting that all of their effects on aldosterone secretion are direct. The data of the present investigations do not, however, completely rule out the possibility that kaliuretic peptide may inhibit potassium-stimulated aldosterone secretion as well as directly inhibiting aldosterone secretion since infusion of 10 meq potassium, which does not change the measured plasma potassium concentration, increases plasma aldosterone concentration by 25% (Takagi et al. 1986). Thus aldosterone secretion appears to be regulated by very small changes in potassium, which are not always measurable with potassium assays (Takagi et al. 1986).

With respect to kaliuretic peptide’s effect on aldosterone secretion, it is important to note that both ANF and kaliuretic peptide are released simultaneously following physiological stimuli such as those induced with central hypervolemia secondary to head-out of water immersion (Vesely et al. 1995). Both ANF and kaliuretic peptide circulate normally in healthy humans, but kaliuretic peptide circulates at 3-fold higher concentrations than ANF (Vesely et al. 1994b, 1995). Since kaliuretic peptide had greater inhibitory effects than ANF in the present investigation, where they were compared at the same
TABLE 1. Effects on plasma potassium concentrations (meg/l) of a 60-min infusion of kaliuretic peptide, long acting natriuretic peptide, vessel dilator or ANF. Atrial peptides were infused at a dose of 100 ng/kg body weight for 60 min (infusion period from 60–120 min). Control subjects received vehicle only. Values are means ± s.d., n=6

<table>
<thead>
<tr>
<th>Peptide</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>80</th>
<th>100</th>
<th>120</th>
<th>150</th>
<th>180</th>
<th>210</th>
<th>240</th>
<th>270</th>
<th>300</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaliuretic peptide</td>
<td>4.24 ± 0.07</td>
<td>4.16 ± 0.05</td>
<td>4.14 ± 0.09</td>
<td>4.08 ± 0.11</td>
<td>4.06 ± 0.11</td>
<td>4.13 ± 0.08</td>
<td>4.17 ± 0.06</td>
<td>4.20 ± 0.05</td>
<td>4.14 ± 0.09</td>
<td>4.09 ± 0.011</td>
<td>4.18 ± 0.04</td>
<td>4.21 ± 0.05</td>
</tr>
<tr>
<td>LANP</td>
<td>4.21 ± 0.06</td>
<td>4.19 ± 0.05</td>
<td>4.18 ± 0.07</td>
<td>4.22 ± 0.04</td>
<td>4.17 ± 0.09</td>
<td>4.15 ± 0.07</td>
<td>4.12 ± 0.09</td>
<td>4.16 ± 0.07</td>
<td>4.12 ± 0.08</td>
<td>4.15 ± 0.06</td>
<td>4.19 ± 0.05</td>
<td>4.22 ± 0.04</td>
</tr>
<tr>
<td>Vessel dilator</td>
<td>4.28 ± 0.07</td>
<td>4.17 ± 0.06</td>
<td>4.19 ± 0.08</td>
<td>4.26 ± 0.04</td>
<td>4.18 ± 0.09</td>
<td>4.22 ± 0.08</td>
<td>4.12 ± 0.12</td>
<td>4.19 ± 0.06</td>
<td>4.12 ± 0.11</td>
<td>4.15 ± 0.08</td>
<td>4.17 ± 0.09</td>
<td>4.24 ± 0.06</td>
</tr>
<tr>
<td>ANF</td>
<td>4.23 ± 0.08</td>
<td>4.19 ± 0.06</td>
<td>4.21 ± 0.09</td>
<td>4.19 ± 0.08</td>
<td>4.22 ± 0.07</td>
<td>4.18 ± 0.09</td>
<td>4.17 ± 0.06</td>
<td>4.22 ± 0.09</td>
<td>4.19 ± 0.04</td>
<td>4.22 ± 0.07</td>
<td>4.18 ± 0.06</td>
<td>4.16 ± 0.11</td>
</tr>
<tr>
<td>Controls</td>
<td>4.25 ± 0.09</td>
<td>4.22 ± 0.07</td>
<td>4.20 ± 0.06</td>
<td>4.18 ± 0.05</td>
<td>4.19 ± 0.04</td>
<td>4.17 ± 0.08</td>
<td>4.13 ± 0.09</td>
<td>4.17 ± 0.08</td>
<td>4.15 ± 0.11</td>
<td>4.20 ± 0.06</td>
<td>4.21 ± 0.05</td>
<td>4.19 ± 0.07</td>
</tr>
</tbody>
</table>

LANP = Long acting natriuretic peptide; ANF = atrial natriuretic factor.

There was no significant difference in plasma potassium concentrations secondary to the infusion of any of these atrial peptides compared to their own baseline values when evaluated by one-way ANOVA.
concentration, it may well be that under physiological conditions where the concentration of kaliuretic peptide in the circulation is 3-fold higher than ANF, its contribution to inhibition of aldosterone may be even more important. It was further demonstrated in the present investigation that kaliuretic peptide inhibits aldosterone secretion for a significantly (P<0.001) longer period than ANF when both were used at the same concentration. Thus, when ANF and kaliuretic peptide act in concert to inhibit aldosterone secretion, ANF's effects on aldosterone secretion cease within 30 min of stopping its infusion while kaliuretic peptide still significantly inhibits aldosterone secretion 3 h after ceasing its infusion. Therefore the inhibition of aldosterone secretion for more than 30 min which occurs after physiological stimuli which cause the release of atrial peptides is not due to ANF but rather to kaliuretic peptide and long acting natriuretic peptide (which also significantly decreased aldosterone secretion for 3 h after cessation of infusion).

Long acting natriuretic peptide (LANP) significantly decreased the plasma and urine concentrations of aldosterone. When the effect of LANP on the secretion of aldosterone from isolated adrenal cells was tested, there was a small decrease in aldosterone secretion but this decrease was not statistically significant (Denker et al. 1990). LANP and vessel dilator were released simultaneously with ANF from isolated perfused atria (Dietz et al. 1991). They are also released simultaneously with ANF in response to a sodium load in vitro (Dietz et al. 1992) and following central hypervolemia in humans (Vesely et al. 1989), their concentrations in the circulation being 10- to 15-fold higher than ANF (Winters et al. 1989). The fact that LANP inhibited aldosterone secretion for a significantly (P<0.001) longer period than ANF plus the fact that LANP circulates at 15-fold higher concentrations than ANF (Winters et al. 1989, Gower et al. 1994) suggests that LANP, along with kaliuretic peptide, may be an important physiological regulator of aldosterone secretion. Vessel dilator had no effect on aldosterone secretion in any of the human subjects in the present investigation. The lack of effect of vessel dilator in vivo is in agreement with...
with the in vitro study where it had no effect on aldosterone secretion from isolated adrenal cells (Denker et al. 1990).

The renin–angiotensin system is modified by a number of factors, one of which is the adrenergic nervous system. Activation of the adrenergic nervous system caused by the mental stress of participating in a study in which the subjects did not know if they were receiving placebo (vehicle only) or one of the active peptides, is the most likely reason why plasma aldosterone increased in the control subjects. The ability of the atrial peptides to inhibit aldosterone secretion in light of the probable same activation of the adrenergic nervous system in the subjects receiving the peptide infusions indicates that their effects are stronger on the renin-aldosterone system than the influence of the adrenergic nervous system on the renin-aldosterone system. In summary, this investigation demonstrates that three peptides derived from the ANF prohormone decrease aldosterone secretion, and that both kaliuretic peptide and long acting natriuretic peptide inhibit aldosterone secretion for a significantly longer period than ANF.

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