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SHORT COMMUNICATION

EFFECT OF PRO-ATRIAL NATRIURETIC PEPTIDES 1–30, 31–67 AND 99–126 ON ANGIOTENSIN II-STIMULATED ALDOSTERONE PRODUCTION IN CALF ADRENAL CELLS

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Summary—Pro-atrial natriuretic peptide (Pro-ANP) is a 126 amino acid peptide from which atrial natriuretic peptide (ANP) (99–126 amino acid) is derived. ANP has potent diuretic, natriuretic and vasodilatory properties. ANP is also a potent direct and indirect inhibitor of aldosterone secretion. The N-terminus of the ANP prohormone containing the peptides 1–30 and 31–67 have been demonstrated to have diuretic, natriuretic and vasodilatory properties.

Dispersed calf zona glomerulosa cells were incubated with angiotensin II (A-II) and increasing concentrations of ANP, ProANP 1–30 and 31–67 to determine if their reported natriuretic activity was mediated through suppression of aldosterone secretion. ANP as reported by many investigators produced a dose-dependent and potent inhibition of A-II-mediated aldosterone secretion. The Pro-ANP 1–30 and 31–67 did not affect A-II-stimulated aldosterone secretion at any of the doses tested. This study shows that the reported natriuretic effect of the fragments is not mediated by inhibition of aldosterone secretion.

INTRODUCTION

Atrial natriuretic peptide (ANP) is formed as a preprohormone of 151 amino acids. Proteolytic cleavage removes a hydrophobic leader segment to form a 126 amino acid prohormone (Pro-ANP). ANP represents the 28 amino acid carboxy-terminal end of the prohormone (99–126) [1]. ANP has potent natriuretic, diuretic and vasodilatory properties [1, 2], and it has been shown to be a potent *in vivo* and *in vitro* inhibitor of aldosterone secretion via a direct effect on the adrenal [3, 4] and indirectly through inhibition of renin release [1, 5].

The presence of dibasic amino acids in the 1–98 N-terminal end of the molecule led to the investigation of other possible proteolytic cleavage products and resulted in the identification of the N-terminal amino acid segments 1–30 (Pro-ANP 1–30) and 31–67 (Pro-ANP 31–67) in the circulation of humans [6, 7]. The relative plasma concentrations of these peptides are much larger than that of ANP [7]. Pro-ANP 1–30 and Pro-ANP 31–67 have potent natriuretic, diuretic and vasodilatory properties, and to activate particulate guanylate cyclase activity similar to ANP [2, 8, 9].

The purpose of the present investigation was to determine if the Pro-ANP 1–30 and Pro-ANP 31–67 inhibited angiotensin II (A-II)-stimulation of aldosterone secretion in dispersed calf zona glomerulosa cells *in vitro*.

EXPERIMENTAL

Most chemicals and reagents were obtained from Sigma Chemical Company (St Louis, Mo.). The [1,2,6,7-^{3}H]aldosterone was purchased from Amersham Corporation (Arlington Heights, Ill.). ANP, Pro-ANP 1–30, Pro-ANP 31–67 were obtained from the American Peptide Company (Santa Clara, Calif.).

Dispersed calf adrenal zona glomerulosa cells

Adrenal cells were obtained from the outer 500 μ slice from calf adrenals and dispersed using collagenase and neutral protease as previously described [10]. Approximately 260,000 cells per tube were suspended in Ham-F-12 media containing 0.5% of bovine serum albumin with the various concentrations of peptides and incubated at 37°C for 2 h. The cells were

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incubated with 10^{-9} M A-II and increasing concentrations of ANP, Pro-ANP 1–30 and Pro-ANP 31–67 (10^{-11}–10^{-8} M). At the end of the incubation period, the cells were separated by centrifugation and the supernatant stored frozen at −20°C. Aldosterone was measured by direct radioimmunoassay using a monoclonal antibody [11]. The experiments were repeated 3 times. Statistical analysis was done using an ANOVA program for the Macintosh Computer (Statview 512: BrainPower Inc., Calabazas, Calif.).

RESULTS AND DISCUSSION

A-II produced a marked stimulation of aldosterone secretion which was inhibited by ANP in a dose-dependent manner (Fig. 1). Inhibition of aldosterone secretion was significant at a concentration of ANP of 10^{-11} M. Neither Pro-ANP 1–30 or Pro-ANP 31–67 inhibited A-II-stimulated aldosterone secretion at any of the doses tested (Fig. 1).

This study showed again that ANP is a potent inhibitor of A-II-mediated aldosterone secretion [3, 4]. Pro-ANP 1–30 and Pro-ANP 31–67 have been shown to have similar natriuretic, diuretic and vasodepressor activity and to stimulate guanylate cyclase [2, 8, 9] as ANP, but they did not have any effect as ANP has on aldosterone secretion. The mechanism by which Pro-ANP 1–30 and Pro–ANP 31–67 exert their natriuretic activity does not involve inhibition of aldosterone secretion. Pro-ANP 1–30 and Pro-ANP 31–67 are structurally very different from ANP and bind separate receptors [12].

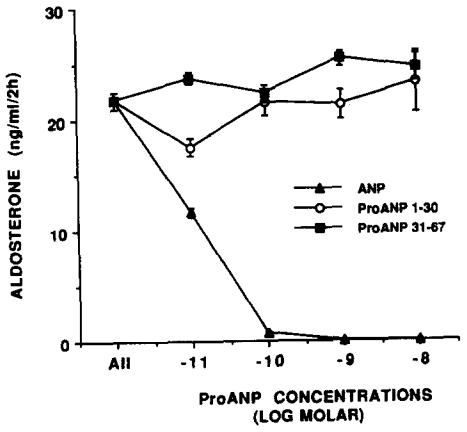

Fig. 1. Effect of pro-atrial natriuretic peptides 1–30 (Pro-ANP 1–30), 31–67 (Pro-ANP 31–67) and ANP on angiotensin II (10^{-9} M)-stimulated aldosterone secretion (mean ± SEM).

The presence of Pro-ANP 1–30 in plasma is unclear since immunoreactive Pro-ANP 1–30 of plasma extracts submitted to gel filtration chromatography elute with the Pro-ANP 1–98 N-terminal fragment peak [7, 13]. Winters *et al.* [7] has shown that immunoreactive Pro-ANP 31–67 from plasma extracts co-eluted with the synthetic peptide using HPLC and Sephadex G-50 gel filtration chromatography indicating that Pro-ANP 31–67 circulates as a distinct molecular entity. Chen *et al.* [14] found that immunoreactive Pro-ANP 1–30 and Pro-ANP 31–67 of a plasma extract subjected to HPLC co-eluted, but the peak was not at the same location as either synthetic peptide, however, no further characterization by sizing or sequencing was performed. The final determination of the molecular species of these immunoreactivies await the elucidation of their structure by sequencing of the plasma extract peaks.

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