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EFFECT OF ATRIAL NATRIURETIC FACTOR (ANF)-RELATED PEPTIDES ON ALDOSTERONE SECRETION BY ADRENAL GLOMERULOSA CELLS: CRITICAL ROLE OF THE INTRAMOLECULAR DISULPHIDE BOND

Lynn Chartier, Ernesto Schiffrin[1], Gaëtan Thibault

Clinical Research Institute of Montreal
110 Pine Avenue West, Montreal, Quebec, Canada H2W 1R7

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We previously demonstrated that synthetic 48-73 atrial natriuretic factor (ANF) (previously called 8-33 ANF) blocked the response of rat adrenal glomerulosa cells to angiotensin II, ACTH and potassium. We have now investigated the effects of natural 43-73 ANF, oxidised synthetic 48-73 ANF and the natural 1-73 ANF on aldosterone output by rat glomerulosa cells. The natural 43-73 ANF and the natural 1-73 ANF were equipotent to 48-73 ANF in inhibiting the stimulation of aldosterone secretion produced by angiotensin II with an IC_{50} of 2×10^{-9}M. Similar results were obtained with ACTH and potassium. After oxidation with performic acid, 48-73 ANF was completely devoid of activity on the response of aldosterone to angiotensin II, ACTH and potassium. We conclude that the intramolecular disulphide bond in 48-73 ANF is critical for maintaining the active conformation of ANF.

Atrial natriuretic factor (ANF) is a peptide isolated from the atria of the rat (1-3) and other species (4-6). It has powerful natriuretic and vasorelaxant properties. Since ANF blocks angiotensin II-induced contraction of vascular strips (7), we have investigated its effect on basal and stimulated aldosterone output by isolated rat adrenal glomerulosa cells. We reported that the synthetic 48-73 ANF (previously called 8-33 ANF) blocks the effect of angiotensin II, ACTH and potassium on aldosterone secretion without change in basal output (8). We have now examined the actions of the natural 43-73 ANF, of oxidised synthetic 48-73 ANF and of the natural 1-73 ANF, on aldosterone secretion by isolated glomerulosa cells.

METHODS

Preparation and incubation of cell suspension

Isolated rat adrenal glomerulosa cells were prepared according to a technique previously described (9). Briefly, Sprague-Dawley rats were

[1] To whom correspondence should be addressed.

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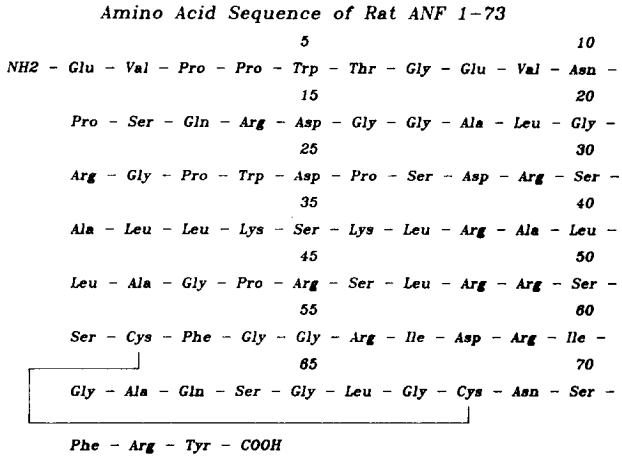

Figure 1: Sequence of 1-73 atrial natriuretic factor (ANF) as reported in reference 10 on which the nomenclature of ANF-related peptides mentioned in this paper is based.

killed by decapitation and adrenal glands were immediately removed and dissected free of fat. The capsules were separated from fasciculata-reticularis by manual compression. Capsules were minced and incubated for 40 min at 37C in medium 199 containing Dispase (Boehringer Mannheim) (80 μg/ml), deoxyribonuclease (DNAase I from bovine pancreas, Sigma) (3 μg/ml), collagenase (Sigma) (1.7 mg/ml) and 2% bovine serum albumin (Miles Laboratories). The cells were dispersed mechanically, filtered through a 100-μ Nitex nylon filter (Tetko, Elmsford, NY) and centrifuged at 200 g for 2 min. The cells were resuspended in F12 nutrient medium (Gibco) containing 0.35% Hepes (Sigma), 0.12% sodium bicarbonate, 0.002% gentamicin (Gibco), and 4% bovine serum albumin (Miles Laboratories). Cells were preincubated for 2 h at 37C in a Dubnoff metabolic shaker. After centrifugation at 200 x g for 2 min they were resuspended in the same medium. The cell suspension was pipetted (0.95 ml) into 1.5 ml Eppendorf tubes containing 0.05 ml of the agents to be tested and dissolved in the same medium. The tubes were capped and incubated for 90 min at 37C. At the end of the incubation, tubes were centrifuged in a Fisher microcentrifuge at 13000 x g for 2 min. The supernatant was decanted into glass tubes and frozen at -20C until assayed for aldosterone. Aldosterone was measured by radioimmunoassay without extraction or chromatography using an antibody kindly provided by Dr. P. Vecsei (University of Heidelberg) as we have previously described (9). Results were expressed as nanograms of aldosterone per 10^6 cells per hour incubation. Synthetic 48-73 ANF was graciously provided by Dr. Ruth Nutt of Merck, Sharp and Dohme Research Laboratory (West Point, Penn.). The natural 43-73 and 1-73 ANF were purified as described (10). Since the final maturation form of ANF is not yet known, this nomenclature is based on the aminoacid sequence of the 73 aminoacid high molecular form of ANF (Fig. 1). Oxidation of 48-73 ANF with performic acid was done as described by Hirs (11).

RESULTS

Synthetic 48-73 ANF inhibited the aldosterone response to angiotensin II as expected with an IC_{50} of 2×10^{-9}M (Fig. 2). Natural 43-73 ANF and 1-73 ANF had identical effects on the aldosterone response to angiotensin II.

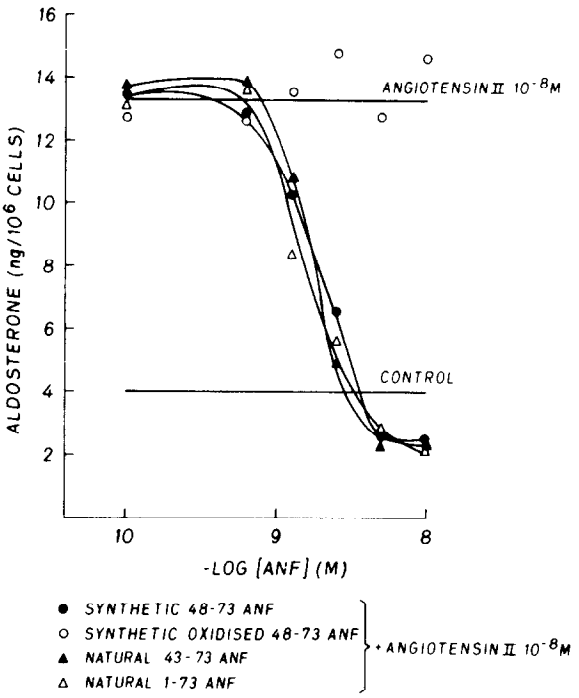

Figure 2: Dose response curve of basal and stimulated aldosterone output to ANF by rat adrenal glomerulosa cells in response to angiotensin II. Results are means of close duplicates in a representative experiment. Similar results were obtained in 4 other experiments.

The oxidised 48-73 ANF had no effect on the response of aldosterone biosynthesis to angiotensin II.

Aldosterone secretion rose to 18.0 ± 1.8 ng/10^6 cells after ACTH 2.9 x 10^{-10}M and to 8.8 ± 0.25 ng/10^6 cells when exposed to 10 mM potassium from a basal output of 0.2 ± 0.1 ng/10^6 cells. Inhibition of the aldosterone response to ACTH (2.9 x 10^{-10}) and potassium (15 mM) was identical for synthetic 48-73 and natural 43-73 ANF. 1-73 ANF was available in small amounts and we only studied it on ACTH-stimulated responses. The IC_{50} for ANF on ACTH and potassium-stimulated aldosterone output was 8 x 10^{-10}M. Oxidised 48-73 ANF was equally ineffective on ACTH and potassium stimulated glomerulosa responses, as found with angiotensin II-induced steroidogenesis.

DISCUSSION

Our results demonstrate that ANF is a powerful inhibitor of stimulated aldosterone secretion by isolated rat glomerulosa cells. The present study

extends our previous observation on 48-73 ANF (8) to the natural compounds 43-73 and 1-73. It is of interest that 1-73 ANF appears to be less potent as a natriuretic agent than the 48-73 ANF (7), while both peptides are equipotent as inhibitors of stimulated aldosterone secretion. This indicates that molecular requirements for interaction with the putative receptor in the kidney and on the adrenal may be different.

The data we report *in vitro* is supported by results we have already obtained *in vivo* which demonstrate that ANF may inhibit aldosterone secretion stimulated by angiotensin II (12). Furthermore, we already have evidence for the existence of specific receptors for ANF in the rat adrenal glomerulosa which are presumable involved in mediating the inhibitory effect of stimulated aldosterone secretion we report (13).

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