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ATRIAL NATRIURETIC PEPTIDE INHIBITS THE STIMULATION OF ALDOSTERONE SECRETION BY ACTH IN VITRO AND IN VIVO

LYNN CHARTIER AND ERNESTO L. SCHIFFRIN

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

ABSTRACT: Previous studies have shown that atrial natriuretic peptide (ANP) inhibits the secretion of aldosterone by isolated adrenal glomerulosa cells stimulated by angiotensin II, ACTH and potassium in vitro and by angiotensin II in conscious unrestrained rats. In this study we investigated further the effects of synthetic ANP on the dose-response curve of aldosterone secretion stimulated by ACTH in vitro. ANP displaced the dose-response curve of aldosterone to ACTH to the right with a significant change in EC_{50}. A similar effect of ANP was reproduced in vivo in conscious unrestrained rats. There was no significant effect of ANP on the corticosterone response to ACTH in vivo. ANP is a potent regulator of aldosterone secretion which may modulate the effects of ACTH on the adrenal in vitro and in vivo.

The major factors which regulate aldosterone secretion are angiotensin II, ACTH and potassium (1, 2). We have reported that synthetic atrial natriuretic peptide (ANP) blocks the response of rat adrenal glomerulosa cells to angiotensin II, ACTH and potassium (3, 4). Atarashi et al. (5) simultaneously reported similar effects on aldosterone production with an atrial extract. We also demonstrated that ANP inhibited the stimulation of aldosterone secretion by angiotensin II in vivo in conscious rats (4). Furthermore, the presence of specific receptors for ANP in the adrenal capsule of the rat has been demonstrated (6).

We have now examined in greater detail the effect of ANP on the dose-response curve of aldosterone secretion stimulated by ACTH in vitro using isolated rat adrenal glomerulosa cells. We have also investigated the effect of ANP on adrenal secretion stimulated by ACTH in vivo in conscious unrestrained rats.

MATERIALS AND METHODS. In vitro experiments. Isolated rat adrenal glomerulosa cells were prepared according to a modification of a technique previously described (7). Sprague-Dawley rats weighing 250 g were killed by decapitation and the adrenal glands were immediately removed and dissected free of fat. The capsules were separated from fasciculata-reticularis by manual compression. The capsules were minced and incubated for 40 min at 37°C in F12 nutrient medium (Gibco) containing deoxyribonuclease (DNAase I from bovine pancreas, Sigma) (3 μg/ml), collagenase (Sigma) (1.7 mg/ml) and 2% BSA (Miles). The cells were dispersed mechanically, filtered through a 100 μm Nitex nylon filter (Tetko) and centrifuged at 200 x g for 2 min. The cells were resuspended in the same medium and preincubated for 2 hr at 37°C in a Dubnoff metabolic shaker in an atmosphere of 95% O_2 and 5% CO_2. After centrifugation they were resuspended in the F12 nutrient medium and incubated in the presence of the agents to be tested for 90 min. At the end of the incubation, the cell suspension was centrifuged and the supernatant decanted into glass tubes and frozen at -20°C until assayed for aldosterone by radioimmunoassay.

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In vivo experiments. Male Sprague-Dawley rats weighing approximately 250 g were kept in individual cages and exposed to light from 0600 to 1800 hr at a temperature of 22°C and relative humidity of 60%. They were fed normal rat chow containing 152 mmol/kg of sodium (Purina), and had access to water ad libitum. Under pentobarbital anesthesia (Nembutal, Abbott) (60 mg/kg ip), a PE-20 polyethylene catheter filled with 5% dextrose in water containing 100 U/ml of heparin (Sodium Heparin, Abbott) was placed in the jugular vein, passed under the skin, and brought out at the scruff of the neck. After 48 hr, the conscious rats were studied between 0900 and 1200 hr. Special care was taken to avoid stress in handling the animals. Rats weighing less than before surgery were not used. ACTH and/or ANP were dissolved in 5% dextrose in water. ANP was infused in a total volume of 0.3 ml over 30 min at a rate of 50 ng per min with a Harvard pump model 600-950. At 15 min before the end of the infusion period, a bolus injection of ACTH was given in a volume of 0.2 ml. At the end of the infusion period, the rats were decapitated and blood was collected from the trunk during the first 5 sec in chilled tubes containing EDTA for determination of plasma renin activity. The rest of the blood was collected on heparin for determination of aldosterone and corticosterone concentration. Blood was immediately centrifuged at 4°C and plasma was separated and stored at -20°C until assayed.

Biochemical methods. Plasma renin activity was measured by RIA of angiotensin I generated during a 2-hr incubation at 37°C and pH 6.5 (8). Plasma aldosterone concentration was measured by RIA after extraction and paper chromatography (9) with an antibody kindly provided by Prof. P. Vecsei (University of Heidelberg) as described (7). Plasma corticosterone concentration was measured by RIA after extraction (9). For in vitro experiments, aldosterone was measured without extraction or chromatography.

ANP was synthetic ANP 101-126 previously called ANF 8-33 or 48-73 (3, 4). It was kindly provided by Dr. R.F. Nutt of Merck, Sharp and Dohme Research Laboratories (West Point, Penn.). ACTH was Cortrosyn (Organon).

Analysis of data. Fitting and analysis of dose-response curves was performed with the computer program ALLFIT based on the four-parameter logistic equation (10). Curves were compared by performing a partial F test on the residual sum of squares after fitting the data with or without constraining a parameter (IC_{50}) to be shared or equal. A significant improvement in the goodness of fit (statistically significant reduction in the residual sum of squares) when the parameter was not shared indicated that this parameter was different for each curve. The deviations of mean values on the fitted curves (residuals) were weighted according to the reciprocal of the variance of the values obtained in individual experiments. The number of degrees of freedom for each fit was obtained by subtracting the number of parameters fitted from the number of data points. The null hypothesis was rejected when $p < 0.05$.

RESULTS. In vitro experiments. The effects of different ANP concentrations on aldosterone output by isolated glomerulosa cells stimulated by ACTH are shown in figure 1. ACTH at concentrations above 30 pM produced a significant increase in aldosterone secretion, with an EC_{50} of 0.3 nM, and a plateau was achieved at 3 nM ACTH. In the presence of 30 pM ANP, the dose-response curve of aldosterone output to ACTH was shifted to the right, with an EC_{50} of 0.9 nM ($p < 0.001$ vs no ANP). At 300 pM ANP, the EC_{50} of aldosterone dose-response curve to ACTH was 1.1 nM ($p < 0.001$). At 3 nM ANP the displacement to the right was more pronounced and the EC_{50} for the aldosterone dose-response curve to ACTH was 1.8 nM ($p < 0.001$). At this concentration of ANP, 100 nM ACTH counteracted the inhibitory effect of ANP only partially and elicited a reduced maximal response compared to that in the absence of ANP.

In vivo experiments. Intravenous bolus injections of ACTH into conscious unrestrained rats at a dose of 1, 3, 10 and 100 ng (Fig. 2) produced the

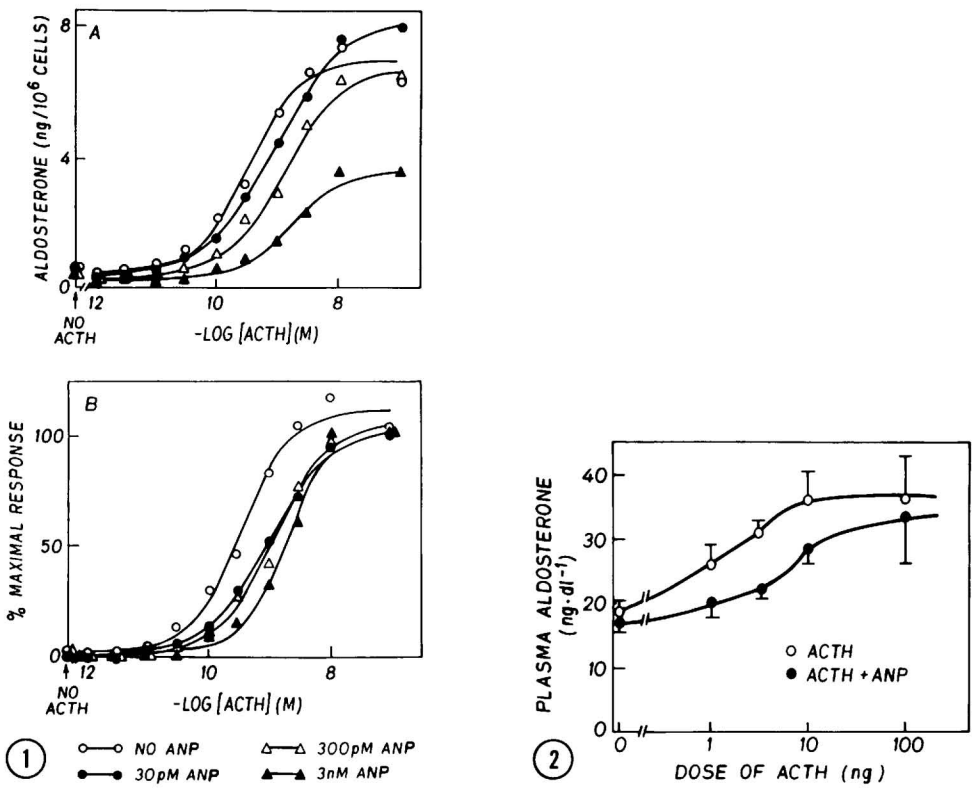

Figure 1: Dose-response of aldosterone secretion by isolated rat glomerulosa cells to ACTH in the presence of ANP. **A.** The results are expressed as nanograms aldosterone per 10^6 cells. The EC_{50} of the aldosterone response to ACTH was 0.3 nM in the absence of ANP (open circles), 0.9 nM in the presence of 30 pM ANP (closed circles) ($p < 0.001$), 1.1 nM in the presence of 300 pM ANP (triangles) ($p < 0.001$) and 1.8 nM in the presence of 3 nM ANP (closed triangles) ($p < 0.001$). **B.** Same data as **A** represented as the percent of maximal response after subtraction of basal aldosterone secretion. 'Results are the means of duplicates in a representative experiment. Similar results were obtained in two additional experiments.

Figure 2: Plasma aldosterone in response to ANP (50 ng per min) at different concentrations of ACTH in conscious unrestrained rats. The curves were fitted by constraining the EC_{50} to be equal for the two curves, in absence and in presence of ANP. This fit provided a significantly higher residual sum squares than if the EC_{50} was allowed to iterate freely, indicating that the EC_{50} of the dose-response curve of plasma aldosterone to ACTH without ANP was significantly different from that in the presence of ANP ($F = 22.9$, $p < 0.003$). The number of animals for each point is given in brackets in Table I.

expected rise in plasma aldosterone concentration after 15 min. The plasma aldosterone response achieved a plateau at 10 ng ACTH. The concurrent iv infusion of ANP, at a dose of 50 ng per min, displaced the dose-response curve of plasma aldosterone to ACTH to the right. A partial F test on the residual sum of squares after a weighted fitting of the data (see analysis of data) from the dose-response curves to ACTH in the presence

Table I

Effect of ACTH and synthetic atrial natriuretic peptide (ANP) infused iv on plasma aldosterone, plasma corticosterone and plasma renin activity of conscious rats

Group		Plasma corticosterone (μg/dl)	Plasma renin activity (ng AI/ml/hr)
Not infused	(9)	10.2 ± 2.7	1.8 ± 0.2
5% dextrose in water	(10)	14.1 ± 2.8	1.5 ± 0.1
ANP	(7)	15.2 ± 3.3	1.4 ± 0.2
ACTH (1 ng)	(10)	20.3 ± 2.1	1.8 ± 0.3
ACTH (1 ng) + ANP	(10)	19.0 ± 3.5	1.9 ± 0.4
ACTH (3 ng)	(8)	24.5 ± 2.3 *	1.8 ± 0.2
ACTH (3 ng) + ANP	(8)	25.9 ± 1.9 *	2.3 ± 0.1
ACTH (10 ng)	(10)	26.7 ± 3.6 *	1.7 ± 0.1
ACTH (10 ng) + ANP	(11)	26.0 ± 3.1 *	2.1 ± 0.2
ACTH (100 ng)	(6)	25.5 ± 2.1 *	3.1 ± 0.4 *
ACTH (100 ng) + ANP	(6)	26.8 ± 1.0 *	2.9 ± 0.3 *

* $p < 0.05$ vs 5% dextrose (control) (one-way analysis of variance followed by a Newman-Keuls test). ANP was infused at 50 ng per min.

and in the absence of ANP showed that the ED_{50} of both curves were significantly different ($F = 22.9$, ($p < 0.003$). It should be noted that at 100 ng, ACTH counteracted the effect of ANP and the maximum response was nearly the same with or without ANP.

ANP had little or no effect on plasma renin activity and corticosterone concentration (Table I).

DISCUSSION. The main consequence of the action of ANP on the effect of ACTH on aldosterone output is a displacement to the right of the EC_{50} of ACTH in vitro and the ED_{50} of ACTH in vivo. This suggests that the in vitro and in vivo effects are mediated by similar mechanisms. On the other hand, the displacement of the EC_{50} of ACTH to the right suggests a competitive antagonism. We have however recently demonstrated the existence of specific receptors for ANP in the adrenal (6). ACTH does not displace ANP from these binding sites. Furthermore, ANP also blocks the effects of angiotensin II and potassium on aldosterone secretion (3-5, 11). We have recently found that in contrast to what we report for ACTH, ANP does not change the EC_{50} of angiotensin II or potassium on aldosterone output, but rather reduces maximal response (unpublished observations). This suggests that ANP, acting on specific receptors, produces simultaneously an apparently competitive antagonism of the action of ACTH and non competitive antagonism of the action of angiotensin II and potassium. Similar effects were reported by Fakunding and Catt (12) with verapamil and have been found by us with nifedipine (unpublished observations). It has been speculated that these calcium channel blockers interfere with the availability of intracellular calcium which acts as a second messenger for angiotensin II and potassium (7, 12, 13) and which may be required for interaction of the ACTH-receptor complex with adenylate cyclase (14). Thus, ANP may act in a way similar to calcium channel blockers and interfere with the calcium requirement for the coupling of the ACTH-receptor complex and the regulatory subunit of adenylate cyclase. This would explain the apparently competitive type of antagonism found even though ANP binds to specific receptors different from ACTH receptors (6).

In this study ANP infusion did not determine changes in plasma renin activity in conscious unrestrained rats. This resembles what we have found in a previous study (4). It is interesting in this respect that other investigators have reported a decrease

in renin secretion in anesthetized dogs (15). Whether this is the result of differences in protocol or species is unclear at present. Corticosterone concentration in plasma and the response to ACTH was not affected either. This agrees with the prior observation that in rat fasciculata we do not detect high affinity receptors for ANP (6).

These results suggest that ANP may regulate aldosterone secretion stimulated by ACTH in vitro and in vivo and suggest a possible mechanism of action of ANP.

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