Atrial natriuretic factor inhibits the early pathway of steroid biosynthesis in bovine adrenal cortex

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We have previously determined that atrial natriuretic factor (ANF) is a potent inhibitor of steroid secretion in cultured bovine zona glomerulosa and fasciculata cells. The present report describes a comparison of the effect produced by ANF on aldosterone, deoxycorticosterone and progesterone secretions by zona glomerulosa cells and on cortisol, corticosterone and progesterone secretions by zona fasciculata cells. The equipotent inhibitory action of ANF on the stimulated secretion of these steroids in both cell types indicates a common site of action prior to progesterone synthesis at which ANF inhibits the steroidogenic pathway.

Atrial natriuretic factor *Adrenal steroid secretion* *Zona glomerulosa cell* *Zona fasciculata cell*

1. INTRODUCTION

We have previously reported that atrial natriuretic factor (ANF) inhibits aldosterone (Aldo) and cortisol (F) secretions by cultured bovine adrenal cells [1]. Because ANF immunoreactivity was detected in plasma [2], it is possible that ANF may be involved in the control of adrenal steroid secretion. Therefore, it was important to determine the site of action of ANF in adrenal regulation. The present studies were performed to investigate the steroid response of cultured bovine glomerulosa and fasciculata cells to ANF. These cells were used because they display a great response and sensitivity to various stimuli such as increased potassium concentration, angiotensin II, ACTH, prostaglandin E_1, forskolin and phorbol ester.

2. MATERIALS AND METHODS

Primary cultures of bovine adrenal zona glomerulosa and zona fasciculata were performed as described [1,3]. After a 3-day culture period, the cells were incubated for 3 h in 1 ml of culture medium supplemented with 0.01% lysozyme, in the presence of tested substances. Each drug treatment was replicated in 4 adjacent plate wells. Aldo, deoxycorticosterone (DOC), corticosterone (B) and F were determined in the incubating media by direct radioimmunoassay, using highly specific antibodies [4]. Progesterone (Prog) was measured by radioimmunoassay after extraction with petroleum ether and separation on miniature Sephadex LH-20 columns [5]. Synthetic ANF (101–126) [6] was generously supplied by Dr R.F. Nutt (Merck, Sharp and Dohme Research, West Point, PA); angiotensin II was purchased from Peninsula (Belmont, CA); ACTH, prostaglandin E_1, forskolin and phorbol ester from Sigma.

3. RESULTS

In zona glomerulosa cells, addition of 10 nM

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angiotensin II, 10 nM ACTH, 1 μM forskolin, 1 μM prostaglandin E_1, 1 μM phorbol ester and 25.5 mM potassium produced a 6.5, 9, 12, 11, 3, and 2.5-fold increase in Aldo production, respectively. Simultaneous incubation of the cells with the maximum inhibitory dose of ANF (10 nM) inhibited the Aldo response to each of the 6 stimulators. Fig.1 shows that the potency of ANF (ED_{50} 30–100 pM) was independent of the stimulated agent.

The experiments shown in fig.2 indicated that ANF also equipotently (ED_{50} 20–90 pM) inhibited hormone-stimulated secretion in fasciculata cells. Addition of 10 nM angiotensin II, 10 nM ACTH and 1 μM prostaglandin E_1, increased F secretion 18, 12 and 9-fold, respectively. However, maximal

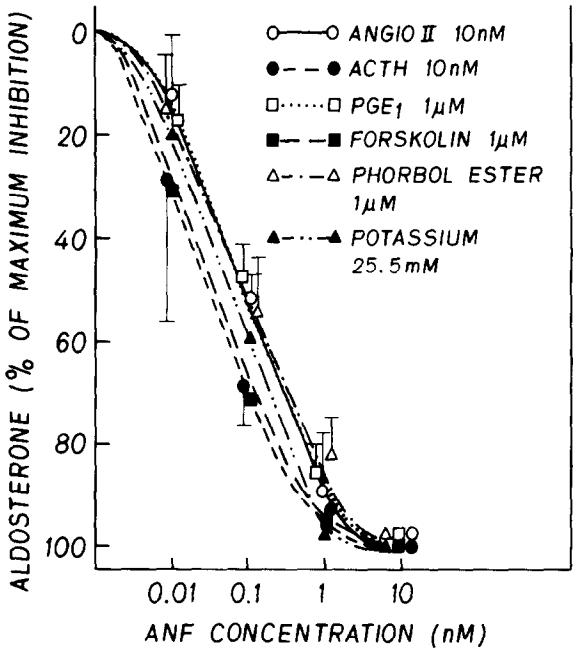

Fig.1. Aldosterone response of stimulated zona glomerulosa cells to different doses of ANF. With angiotensin II, ACTH, prostaglandin E_1, forskolin, phorbol ester and potassium the increase of basal Aldo secretion (1.0 ± 0.1 pmol/10^6 cells per 3 h) was 6.5, 9, 12, 11, 3 and 2.5-fold, respectively. In the presence of the maximum inhibitory dose of ANF (10 nM), the stimulation was decreased by 40, 40, 64, 45, 55 and 65%, respectively. Data are presented as percent of the maximum inhibition.

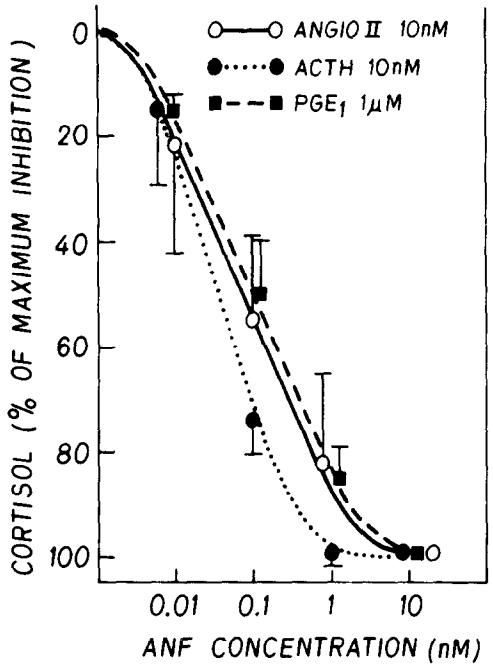

Fig.2. Cortisol response of stimulated zona fasciculata cells to different doses of ANF. With angiotensin II, ACTH and prostaglandin E_1 the increase of basal F secretion (21 ± 4 pmol/10^6 cells per 3 h) was 18, 12 and 9-fold, respectively. ANF produced its maximum inhibitory effect at 10 nM, with a decrease of the stimulated F secretion by 18, 22 and 29%, respectively. Data are presented as percent of the maximum inhibition. Each result is the mean ± SE from 2–3 experiments.

inhibition of hormone-stimulated F secretion by ANF was only between 18 and 29%.

In addition to the inhibition of Aldo and F, there was a marked fall in DOC, B and Prog secretion in these adrenal cell cultures. In the presence of 10 nM ANF, the prostaglandin E_1-stimulated Prog secretion was reduced by 56% in zona glomerulosa and by 53% in zona fasciculata cells; a similar inhibition was observed in DOC and B production (table 1). ANF also displayed the same potency (ED_{50} 50–100 pM) as inhibitor of prostaglandin E_1-stimulated production of all steroids tested (fig.3).

Fig.4 shows that ANF inhibits non-competitive-

Table 1

Effect of ANF (10 nM) on prostaglandin E_1 (1 μM) stimulated steroid secretion in bovine adrenal cell cultures

	Control	ANF	% change
Zona glomerulosa			
Progesterone (n = 3)	167 ± 54	74 ± 20[a]	−56
Deoxycorticosterone (n = 5)	77 ± 22	35 ± 11[a]	−55
Aldosterone (n = 4)	10 ± 2.6	3.8 ± 0.6[a]	−64
Zona fasciculata			
Progesterone (n = 3)	97 ± 37	46 ± 19[a]	−53
Corticosterone (n = 3)	565 ± 90	259 ± 44[a]	−54
Cortisol (n = 3)	191 ± 41	135 ± 34[a]	−29

Steroid secretion of cell cultures is expressed in pmol/10^6 cells per 3 h (mean ± SE). In glomerulosa cell cultures the basal secretion of Prog, DOC and Aldo was 16 ± 3, 18 ± 6 and 1 ± 0.1 pmol, respectively. In fasciculata cultures the basal secretion of Prog, B and F was 11 ± 0.5, 19 ± 4 and 21 ± 4 pmol, respectively. n = number of experiments. [a] $p < 0.05$; [b] $p < 0.01$

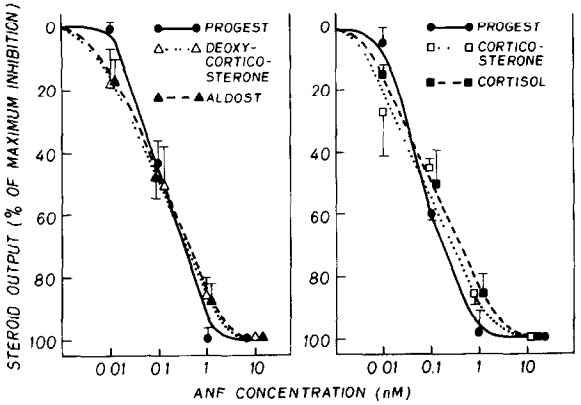

Fig.3. Progesterone, deoxycorticosterone and aldosterone response of prostaglandin E_1-stimulated glomerulosa cells (left), and progesterone, corticosterone and cortisol response of prostaglandin E_1-stimulated fasciculata cells (right) to different doses of ANF. Steroid output is expressed as percent of the maximum inhibition. Each result is the mean ± SE from 2–3 experiments.

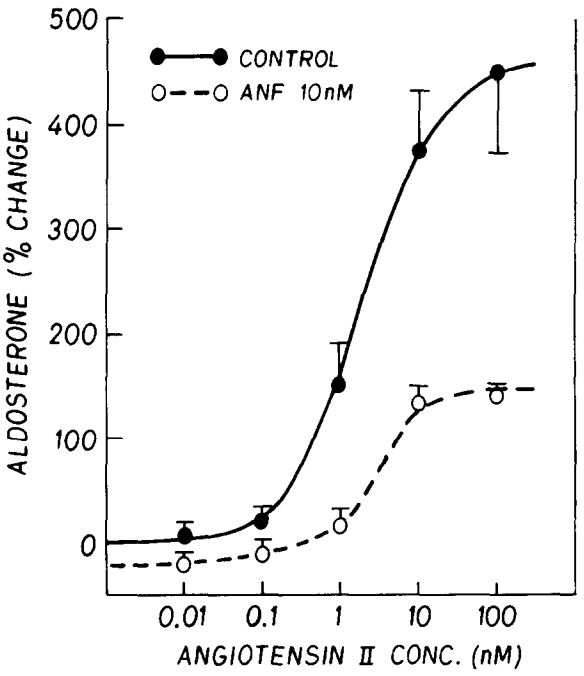

Fig.4. Angiotensin II dose-response curve in the absence and presence of ANF (10 nM). Each result is the mean ± SE from 4 experiments.

ly, hormone-stimulated steroidogenesis. Addition of 10 nM ANF markedly reduced the maximum Aldo response, without changing the ED_{50} with respect to angiotensin II stimulation.

4. DISCUSSION

These studies confirm that ANF is a potent inhibitor of steroid secretion of both zona glomerulosa and zona fasciculata cells [7–9]. The observation that ANF was equipotent in reducing Aldo, DOC and B secretion in glomerulosa cells may indicate that inhibition prior to progesterone is the primary site of action of ANF in zona glomerulosa cells. This mechanism would also explain the results of the experiments noted in fig.4. These data indicate that ANF reduces the maximum level of stimulation of Aldo secretion by angiotensin II, without altering its potency (ED_{50}), which suggests that ANF removes a factor from the process of Aldo synthesis.

It is remarkable that the effect of ANF on Prog secretion of both glomerulosa and fasciculata cells was nearly identical. By analogy it would appear that ANF inhibited steroidogenesis in fasciculata cells through the same mechanism as in glomerulosa cells. This interpretation is supported by the findings that ANF displayed an equivalent potency (ED_{50}) in inhibiting the secretion of Prog, B and F. However, the suppression of F secretion with the maximum inhibitory dose of ANF was only 18–29% while Prog and B secretions showed a more prominent suppression. If ANF acts as an inhibitor of the conversion of pregnenolone to Prog in zona fasciculata cells, at least some part of its inhibitory action on F secretion (but not on B and Prog secretions) would be obscured by the synthesis of F through an alternative pathway (pregnenolone – 17-OH-pregnenolone – F). This explanation is compatible with the view that 17-OH-pregnenolone is an important intermediate in F synthesis [10]. Alternatively, the inhibition of the early biosynthetic pathway by ANF may occur prior to pregnenolone formation. In this case the different suppression of F and B may be due to the possibility that under circumstances when the formation of early biosynthetic precursors is inhibited by ANF, the zona fasciculata utilizes disproportionally high amounts of precursor for F synthesis at the expense of B.

In summary, our results indicate that ANF has an equipotent inhibitory action on steroid secretion of both glomerulosa and fasciculata cells in vitro. The main site of action of ANF appears to be a biosynthetic step prior to Prog formation. The significance of these data in the physiological control of adrenal steroid secretion remains to be evaluated.

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