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SITES OF ACTION OF ANGIOTENSIN II, ATRIAL NATRIURETIC FACTOR AND GUANABENZ, ON ALDOSTERONE BIOSYNTHESIS

MICHÈLE BROCHU,[1] HUY ONG[1,3] and ANDRÉ DE LÉAN[1,2]*

[1]Laboratory of Molecular Pharmacology, Clinical Research Institute of Montréal, 110 Pine Avenue West, Montréal, Québec, Canada H2W 1R7 and [2]Department of Pharmacology and [3]Faculty of Pharmacy, University of Montréal, Montréal, Québec, Canada H3C 3J7

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Summary—It is well known that atrial natriuretic factor (ANF) inhibits aldosterone biosynthesis. Recent studies showed that amiloride can also inhibit adrenal steroidogenesis. Since the antihypertensive agent, guanabenz, is structurally related to amiloride, we have examined its action on aldosterone biosynthesis. The aim of this work was to localize the sites of action of angiotensin II (AII) and of ANF on steroidogenesis and to compare the effects of guanabenz to ANF. Trilostane, an inhibitor of 3β-hydroxysteroid dehydrogenase was used to separately study the early and late pathways of aldosterone biosynthesis. The different steps of steroidogenesis are stimulated by AII. ANF inhibits the formation of pregnenolone, the steps between progesterone and deoxycorticosterone, deoxycorticosterone and corticosterone and finally, corticosterone and aldosterone with ED_{50} of 114 ± 17, 199 ± 90, 14 ± 3 and 92 ± 34 pM of ANF, respectively, and around 70% of inhibition. These steps are also inhibited by guanabenz with ED_{50} of 66 ± 17 μM for the formation of pregnenolone, 1.6 ± 1.3, 3.3 ± 1.7 and 29 ± 4 μM for the last 3 steps. The percentage of inhibition by guanabenz was at least 80% for all the steps except for progesterone to deoxycorticosterone which is <35%. These results indicate that the major site of action of both AII and ANF could be at the level of intracellular signal transduction for the activation of mitochondrial steroidogenic enzymes or for the transport of steroids to mitochondria. We also showed that guanabenz mimics the inhibitory effects of ANF. This study with guanabenz suggests that it might be a prototype for a new family of antihypertensive agents.

INTRODUCTION

Angiotensin II (AII) is known to enhance aldosterone biosynthesis. McKenna *et al.* [1] showed that when AII was added along with deoxycorticosterone in bovine adrenal zona glomerulosa cell suspensions, the conversion of deoxycorticosterone to aldosterone was enhanced. They believed that this peptide stimulates aldosterone biosynthesis through at least two independent sites, one early and one late in the biosynthetic pathway. However, another group [2] suggested that the late pathway of aldosterone production is not affected by AII in bovine cells but no data was shown. It has been demonstrated that in intact and hypophysectomized rats, prolonged infusion of AII increased the enzymes of the early and late pathway of aldosterone biosynthesis. The stimulatory effects of AII on 21-hydroxylase and on 11-hydroxylase were most obvious in hypophysectomized rats, when basal levels of the enzymes were decreased in the absence of ACTH [3]. The effects of AII on aldosterone biosynthesis was to increase the rate of *P*450scc and the conversion of corticosterone to aldosterone [4].

Atrial natriuretic factor (ANF) is a peptide hormone that acts on a specific receptor to inhibit aldosterone secretion [5–12]. The step of steroidogenesis on which this hormone produces its effect is not well known. Campbell *et al.* [5] showed that ANF inhibited conversion

*To whom correspondence should be addressed at: Department of Pharmacology, University of Montréal. Molecular Pharmacology Laboratory, Clinical Research Institute.

Abbreviations: Aldosterone, 11β,21-dihydroxy-3,20-dioxo-4-pregnene-18-al; corticosterone, 11β,21-dihydroxy-4-pregnene-3,20-dione; deoxycorticosterone, 21-hydroxy-4-pregnene-3,20-dione; progesterone, 4-pregnene-3,20-dione; pregnenolone, 3β-hydroxy-5-pregnene-20-one; cholesterol, 5-cholesten-3β-ol; ED_{50}, effective dose to give 50% maximal activity; AII, angiotensin II; *P*450scc, cholesterol side chain cleavage enzyme; trilostane, 4,4,17-trimethyl,17β-hydroxy-3-oxo-androst-5-ene-2α-carbonitrile; guanabenz, 1-(2,6-dichlorobenzylideneamino)guanidine.

of corticosterone to aldosterone in rat adrenal cells when this step was stimulated with AII. In cultured human adrenal cells, it is suggested that ANF inhibits aldosterone production at the early pathway of steroidogenesis, i.e. before the formation of pregnenolone from cholesterol [6]. In dispersed fetal zone cells of the human fetal adrenal glands, inhibition of cortisol and dehydroepiandrosterone-sulfate suggested that the action of ANF was mediated by inhibition of cholesterol side chain cleavage enzyme or the transfer of cholesterol to the mitochondrion [7]. In dispersed rat adrenal glomerulosa cells, ANF inhibits cholesterol side chain cleavage enzyme activation by potassium, AII and ACTH and the activation of the corticosterone methyl oxidase enzyme complex by potassium [8]. Aldosterone inhibition by ANF in bovine adrenal glomerulosa cell suspensions was localized to the early pathway of steroidogenesis, to a step before cholesterol side chain cleavage [9]. In a previous study, we have shown that ANF inhibits aldosterone biosynthesis in cultured bovine zona glomerulosa cells at the early pathway prior to progesterone [10].

We have already demonstrated that amiloride potentiates ANF interaction with its receptor and that, guanabenz, a non peptidic compound, which is an antihypertensive agent with primarily α2-adrenergic agonist properties elicits this enhancing effect [13]. Amiloride at high concentrations directly inhibits hormone-induced aldosterone secretion at high concentrations [13]. Since guanabenz is structurally related to amiloride with its guanidium moiety connected to an aromatic ring, it was interesting to compare its effect on aldosterone biosynthesis.

The aim of this work was to localize first the sites of stimulation of AII and second, the sites of action of ANF on steroidogenesis compared to the effect of guanabenz.

MATERIALS AND METHODS

Primary cell culture

Primary cultures of bovine adrenal zona glomerulosa were performed as described [14]. In brief, bovine adrenals were obtained from a local slaughterhouse. The glands were cleaned of fat, and a 0.5 mm layer containing the capsule and zona glomerulosa was dissected with a scalpel. The cells were dispersed in Ham's F12 medium (Gibco Labs Inc., Burlington, Ontario) with 0.2% collagenase (Sigma, St Louis, MO), 0.02% DNase type I (Sigma) and 0.2% BSA (Sigma). Washed cells were resuspended in Ham's F12 medium with 10% horse serum (Gibco Labs Inc.), 2% foetal calf serum (Gibco Labs Inc.) and plated in 24 well plates (10^6 cells/well in 1 ml).

Cell stimulation

After 3 days in culture, the cells were washed twice with warm Ham's F12 without serum. Quadruplicate cell culture wells were then stimulated for 3 h with various agents added to fresh serum-free Ham's F12 medium containing 0.02% lysosyme (Sigma). At the end of the incubation period, the medium was quickly removed and frozen at $-20°C$ until assayed for steroids determination.

The formation of pregnenolone was measured in the presence of trilostane (WIN 24,540 from Sterling–Winthrop Research Institute, NY), an inhibitor of 3β-hydroxysteroid dehydrogenase (3β OHSD). When present at a concentration of 0.1 μM, this compound quantitatively inhibited the formation of progesterone from pregnenolone and allowed an accumulation of pregnenolone in the incubation media. Although apparently complete, this inhibition of progesterone production might leave some low residual 3β OHSD activity. With this inhibitor, it was also possible to study the later steps of aldosterone biosynthesis and the actions of the individual stimulating or inhibiting factors upon conversion of added steroid precursors such as progesterone, corticosterone or deoxycorticosterone. These precursors were added at the concentration found in the medium when 10 nM of AII and no inhibitor were used, i.e. 5 nM for progesterone, 10 nM for deoxycorticosterone and 100 nM for corticosterone. The addition of trilostane did not affect the activity of *P*450scc [15]. In the first part of the study, i.e. Figs 2 and 3, AII was used at 100 nM. With the study of the effect of increasing concentration of AII, we observed that 100 nM was a supramaximal concentration. So, for the next part of the study, we used 10 nM of AII.

Measurement of steroids

Aldosterone in cell culture medium was directly measured by RIA as already described [16]. To evaluate the proportion of fasciculata cells, cortisol was measured by RIA [14] and this revealed a contamination $< 10\%$. Pregnenolone, progesterone, deoxycorticosterone

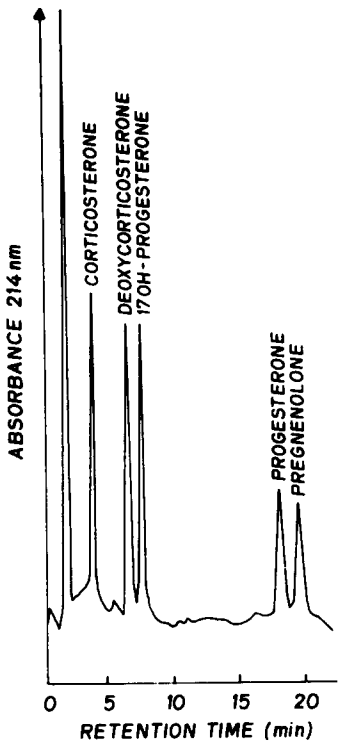

Fig. 1. Elution pattern of steroids on a Nova-Pak C18 column at 45°C using water–acetonitrile–methanol (49:32:19) as eluent, with a flow rate of 0.8 ml/min.

and corticosterone were separated by HPLC. HPLC was carried out with a Varian Vista 5500 equipped with a 2050 u.v. wavelength detector Varian and connected to a collector LKB 2212, and a Pharmacia recorder Rec-481. Absorbance was monitored at 214 nm, 0.04 AUFS. Steroids were resolved on a Nova-Pak C18 column (3.9 mm × 15 cm) (Waters, Montréal, Quebec) at 45°C with a flow rate of 0.8 ml/min, using a solvent system composed of water–acetonitrile–methanol (49:32:19). Each run was performed within 25 min. The elution pattern is shown in Fig. 1.

Pentanophenone was used as internal standard. A 200 μl aliquot of cell culture medium was injected without extraction. To remove contaminants in the cell culture medium, a precolumn Resolve CN (Waters guard-Pak) was inserted into the HPLC system immediately before the analytical column. The concentration of steroids were too low to be detected by u.v., so they were measured by RIA. Progesterone was measured by RIA using iodinated progesterone and precipitation of the bound fraction with polyethylene glycol 12% containing 1/500 anti-rabbit-γ-globulin (Bio-Méga, Montréal, Québec). The RIA for the three other steroids used tritiated standard and charcoal extraction of unbound steroid. The specificity of the antiserum to the four steroids is documented in Table 1, which indicates the percentage of cross reactivity of other steroids with the antiserum, estimated from the values at 50% displacement of tracer binding.

The sensitivity of the whole analytical procedure was determined by the sensitivity of the antibody, the lowest detectable levels of steroids were 55, 9, 16 and 92 fmol/tube for pregnenolone, progesterone, deoxycorticosterone and corticosterone, respectively.

The analytical recovery for the whole method, i.e. separation and RIA of steroids added to cell culture medium, is $95 \pm 12\%$ ($X \pm$ SEM) for pregnenolone, $83 \pm 16\%$ for progesterone, $86 \pm 17\%$ for deoxycorticosterone, $88 \pm 14\%$ for corticosterone.

The coefficient of variation was determined by the measurements ($n = 15$) of steroids in cell culture medium for the whole method. The coefficients of variation of the within-assay and the between-assay were, respectively, 16 and 18% for pregnenolone, 18 and 27% for progesterone, 12 and 16% for deoxycorticosterone, and 14 and 17% for corticosterone.

Materials

All steroids were from Sigma. Anti-pregnenolone, anti-progesterone, anti-deoxycorticosterone, anti-corticosterone, anti-cortisol and

Table 1. Specificity of antiserum to corticosterone, deoxycorticosterone, progesterone and pregnenolone (percent cross-reaction)

Steroid	Anti-corticosterone	Anti-deoxycorticosterone	Anti-progesterone	Anti-pregnenolone
Pregnenolone	0.2	0.7	3.1	100
Progesterone	26	12	100	3.6
17 Hydroxyprogesterone	1.5	<0.001	7.3	0.2
Corticosterone	100	0.4	3.4	<0.02
Deoxycorticosterone	28	100	13	0.3
Deoxycortisol	1.8	0.7	0.3	<0.02
Cortisol	3.5	<0.001	0.002	<0.02
18 Hydroxydeoxycorticosterone	8.5	0.05	0.008	<0.02
18 Hydroxycorticosterone	21	<0.001	0.005	<0.02
Aldosterone	16	<0.001	0.005	<0.02

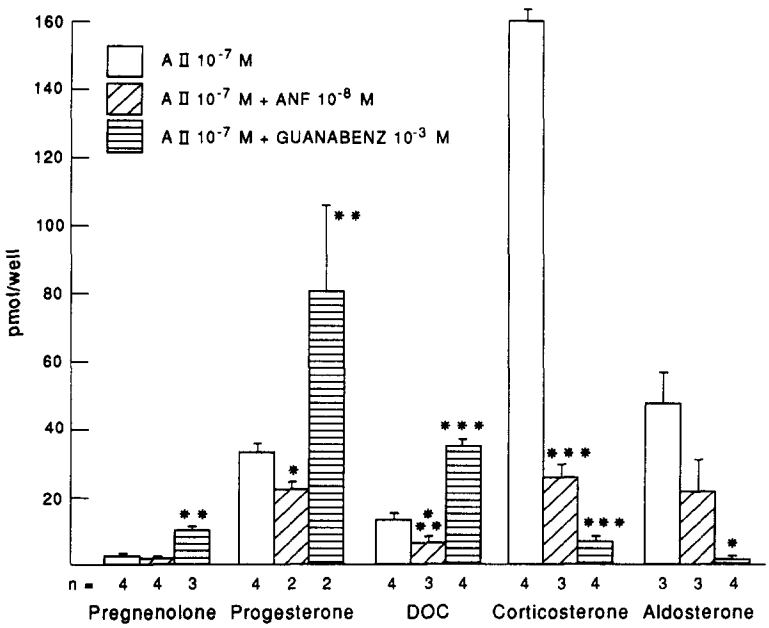

Fig. 2. Effect of ANF and guanabenz on AII-stimulated (10^{-7}) steroidogenesis in bovine adrenal zona glomerulosa cells. No inhibitor was added. DOC = deoxycorticosterone. The number of experiments for each condition is given on the abcissa (10^6 cells/well). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs AII (10^{-7} M).

[^{125}I]progesterone-12(-*o*-carboxymethyl)-oxime tyrosine methyl ester were gifts from Dr Alain Bélanger (Centre Hospitalier, de l'Université Laval, Ste-Foy, Québec). Tritiated pregnenolone, tritiated corticosterone, tritiated deoxycorticosterone and tritiated cortisol were obtained from Amersham (Oakville, Ontario). AII and ANF were purchased from IAF Biochem (Laval, Québec) and guanabenz from Wyeth Labs (Philadelphia, PA).

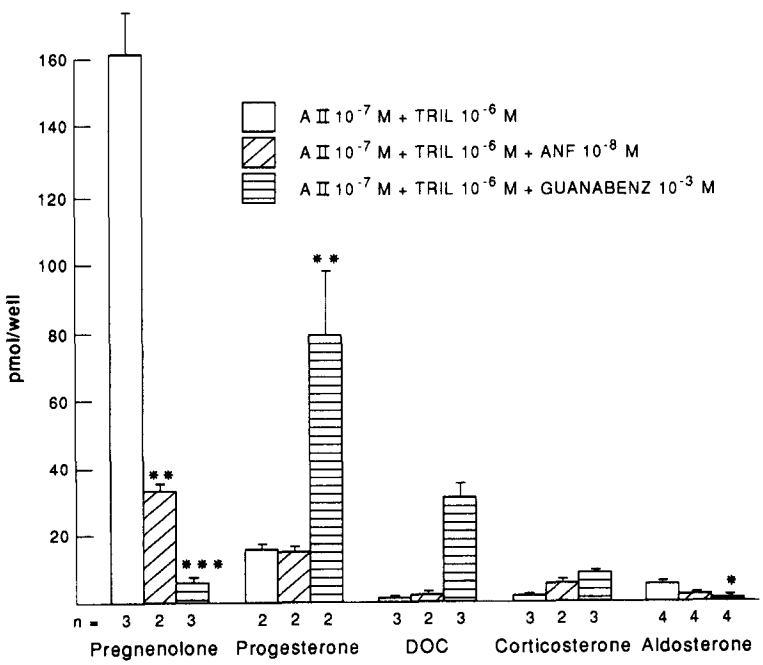

Fig. 3. Effect of ANF and guanabenz on AII-stimulated (10^{-7} M) steroidogenesis in presence of trilostane (10^{-6} M). No precursor was added. DOC = deoxycorticosterone. The number of experiments for each condition is given on the abcissa (10^6 cells/well). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs AII (10^{-7} M) + Tril 10^{-6} M.

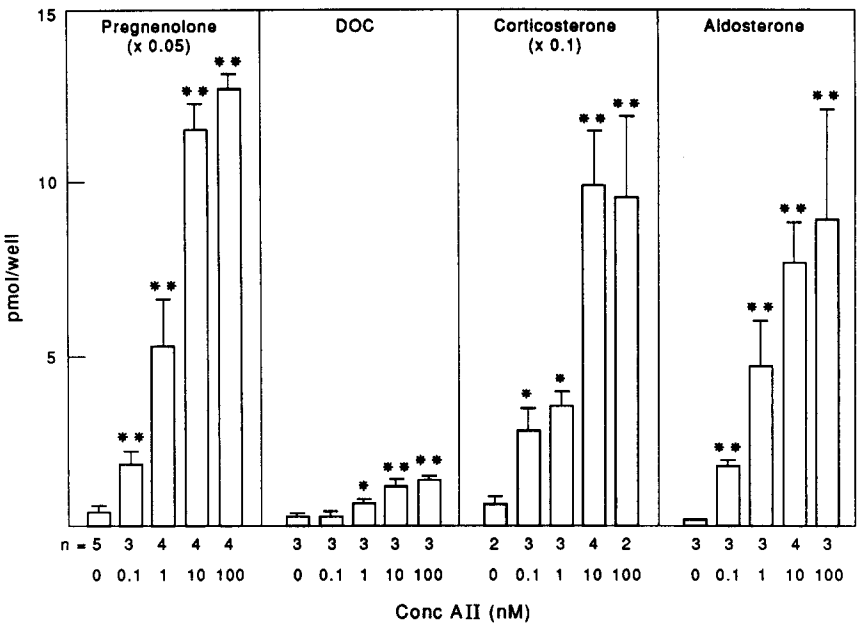

Fig. 4. Stimulatory effect of AII on the different steps of aldosterone biosynthesis. The late steps are separated by using trilostane 10^{-7} M. Precursors such as progesterone (5 nM), deoxycorticosterone (10 nM) or corticosterone (100 nM) were added respectively for the measurement of deoxycorticosterone, corticosterone or aldosterone (10^6 cells/well). $^*P < 0.05$, $^{**}P < 0.01$ vs basal level (0 M AII).

Data analysis

For the first part of the paper (i.e. Table 2, Figs 2–4), the values were compared using Student's t-test. The percentage of inhibition of ANF or guanabenz was calculated as follows:

$$\frac{C_{AII} - C_{inh}}{C_{AII} - C_{basal}} \times 100.$$

Where C_{AII} is the steroid levels in the presence of AII (10 nM), C_{inh} is the steroid levels in the presence of AII (10 nM) and ANF (10 nM) or guanabenz (10 mM), and C_{basal} is the steroid basal levels.

Dose–response curves were analyzed by weighted nonlinear least squares regression using a four parameter logistic equation. This method provides estimates of basal and maximal responses, ED_{50} and slope factor for each dose–response curve. The ED_{50} values for dose–response curves were compared using partial F-test [17].

Results are expressed as average response and SEM of quadruplicate cell culture wells from at least 2 experiments (number given in parentheses).

RESULTS

Effect of ANF and guanabenz on aldosterone biosynthesis

The basal levels for pregnenolone, progesterone, deoxycorticosterone, corticosterone and aldosterone were 4.4 ± 0.6 ($n = 11$), 18.5 ± 1.9 ($n = 10$), 2.7 ± 0.3 ($n = 11$), 15.4 ± 1.5 ($n = 11$) and 5.5 ± 0.9 ($n = 12$) pmol/well, respectively. All these levels (except pregnenolone) increased significantly ($P < 0.001$) in the presence of AII (10^{-7} M). ANF and guanabenz inhibited steroidogenesis in bovine adrenal glomerulosa cells stimulated by AII (10^{-7} M) (Fig. 2), while there was no inhibition of the basal level (data not shown). ANF and guanabenz inhibited the products of the late pathway, i.e. corticosterone ($P < 0.001$) and aldosterone ($P < 0.2$ and $P < 0.05$, respectively). The effect of guanabenz is more pronounced. In addition, ANF inhibited the early pathway (progesterone $P < 0.05$, deoxycorticosterone $P < 0.001$). However, in contrast with ANF, the addition of guanabenz lead to the accumulation of the products of the early pathway pregnenolone ($P < 0.005$), progesterone ($P < 0.05$) and deoxycorticosterone ($P < 0.001$). In order to distinguish the effects of these inhibitory agents on the early and the late pathways, we used trilostane to document both steps separately, as shown in Fig. 3. Accumulation of angiotensin-stimulated pregnenolone production was observed because the formation of progesterone was blocked. The stimulated levels of progesterone, deoxycorticosterone, corticosterone and aldosterone were low since no precursor was added in the medium. An inhibition by ANF ($P < 0.005$) and guanabenz

($P < 0.001$) on the early pathway of aldosterone biosynthesis was observed. Even in the absence of precursor, ANF ($P < 0.1$) and guanabenz ($P < 0.05$) inhibited aldosterone. Interestingly, in the presence of guanabenz, we observed an accumulation of the intermediate products, i.e. progesterone ($P < 0.1$) and deoxycorticosterone ($P < 0.005$) (Fig. 3), similar to that in the absence of trilostane (Fig. 2).

Effect of AII on aldosterone biosynthesis

When trilostane was added, the addition of precursor allowed the study of late steps. The different steps of steroidogenesis were stimulated by AII as shown in Fig. 4. With 0.1 nM of AII, all the steps except the formation of deoxycorticosterone from progesterone were stimulated significantly. At 1 nM of AII, all the steps were stimulated.

The basal levels for the next experiments (inhibition by ANF or guanabenz and the presence of 10^{-7} M of trilostane) were 10 ± 3 pmol/well for pregnenolone, 0.26 ± 0.04 for deoxycorticosterone, 5.8 ± 1.2 for corticosterone and 0.09 ± 0.003 for aldosterone.

Effect of ANF on aldosterone biosynthesis

The inhibition by ANF is observed in Fig. 5, ANF inhibits the formation of pregnenolone, with an ED_{50} of 114 ± 17 pM ($n = 5$). When progesterone was added as precursor, there is an inhibition of deoxycorticosterone with an ED_{50} of 199 ± 90 pM of ANF ($n = 3$). If deoxycorticosterone is used, ANF inhibits the formation of corticosterone with an ED_{50} of 14 ± 3 pm

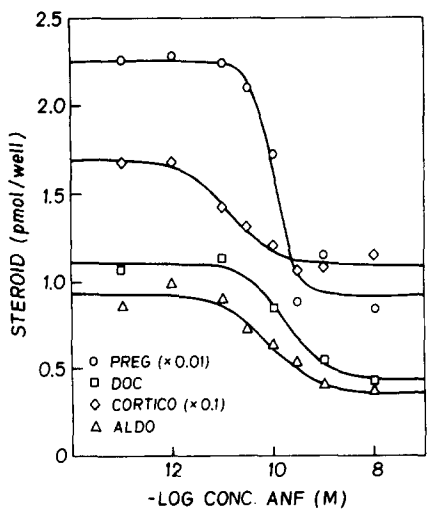

Fig. 5. Effect of ANF on the different steps of AII-stimulated (10^{-8} M) aldosterone biosynthesis. The late steps are separated by using trilostane 10^{-7} M.

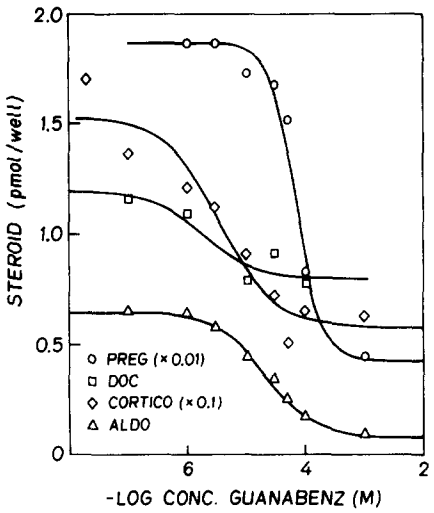

Fig. 6. Effect of guanabenz on the different steps of stimulated aldosterone biosynthesis. The late steps are separated by using trilostane 10^{-7} M. Precursors such as progesterone (5 nM), deoxycorticosterone (10 nM) or corticosterone (100 nM) were added respectively for the measurement of deoxycorticosterone, corticosterone or aldosterone (10^6 cells/well).

($n = 2$). The inhibition curve of aldosterone by ANF with corticosterone as precursor is shown with an ED_{50} of 92 ± 34 pM ($n = 3$).

The ED_{50} of the inhibition curve by ANF for the transformation of deoxycorticosterone to corticosterone is significantly different from the other ED_{50} values (early phase, $P = 0.024$; transformation of progesterone to deoxycorticosterone, $P = 0.011$; corticosterone to aldosterone, $P = 0.013$).

The percentage of inhibition by ANF was around 70% for all the steps.

Effect on guanabenz on aldosterone biosynthesis

The inhibition curve of pregnenolone by guanabenz is shown in Fig. 6, with an ED_{50} of $66 \pm 17\ \mu$M ($n = 5$).

In the presence of trilostane and precursor, all the late steps are inhibited by guanabenz. The ED_{50} for the step from progesterone to deoxycorticosterone ($n = 3$), from deoxycorticosterone to corticosterone ($n = 2$) and finally from corticosterone to aldosterone ($n = 3$) are, respectively, 1.6 ± 1.3, 3.3 ± 1.7 and $29 \pm 4\ \mu$M.

The ED_{50} for the inhibition by guanabenz of the formation of corticosterone from deoxycorticosterone is significantly different from the ED_{50} for the early phase ($P = 0.005$) and from corticosterone to aldosterone ($P = 0.001$) but not from progesterone to deoxycorticosterone. The ED_{50} for the inhibition by guanabenz of the early phase is also significantly different

from that for the late pathway (progesterone to deoxycorticosterone, $P = 0.02$; corticosterone to aldosterone, $P = 0.013$). However, the percentage of inhibition by guanabenz (10^{-3} M) was at least 85% for the early step, 100% for the two last steps, but for the step from progesterone to deoxycorticosterone, it was 35%.

DISCUSSION

We have shown that AII stimulates the different steps of aldosterone biosynthesis. There is no controversy that AII stimulates the early phase but there has been disagreement for the last steps. The control of steroid secretion by AII might differ across species as suggested by Quinn and Williams [18]. This could explain the difference between rat and bovine cells. While Aguilera *et al.* [3] observed stimulatory effects of AII on the step progesterone to deoxycorticosterone in rat cells, Kramer *et al.* [4] did not. The preparation of microsomal protein and the incubation conditions differ between the two groups. Our results agree with those of Aguilera's group even if the techniques and the species were different.

It has been shown that ANF inhibits production of pregnenolone in suspensions of bovine adrenal glomerulosa cells at the basal state and also in the AII-stimulated state and that the late segment of aldosterogenesis was unaffected by ANF or AII [2]. In this paper, no inhibition by ANF on the basal level was observed and the late pathway was inhibited by ANF and stimulated by AII. Methodological differences could be the source of the discrepancy since Elliot and Goodfriend [2] utilized 100-fold higher concentrations of progesterone, thereby allowing for ANF to be overcome by the high dose of progesterone. Differences are shown between cultured cells and cell suspension. However, Campbell *et al.* [5] found in rat suspension of adrenal glomerulosa cells no effect of ANF on the basal level and an increase by AII (2 nM) of the synthesis of pregnenolone as well as the conversion of corticosterone to aldosterone and that atriopeptins act at both the early and late pathways of aldosterone biosynthesis.

The major consideration in the present study was the localization of the sites in the aldosterone biosynthetic pathway at which ANF and guanabenz produced their effects. ANF and guanabenz inhibit the different steps of aldosterone biosynthesis and with different potencies. The step between deoxycorticosterone to corticosterone seems the most sensitive. The higher inhibitory potency of guanabenz for this step combined with some low residual activity of cholesterol side chain cleavage could explain the accumulation of intermediates, e.g. progesterone, deoxycorticosterone (Figs 2 and 3). The fact that they have different potencies for the late and early pathway might suggest a mechanism for regulating steroidogenesis. All the studied steps are mediated by a *P*450 so AII, ANF and guanabenz could have their action on *P*450. The major site of action could be on mitochondrial *P*450 since small inhibition or high ED_{50} were observed for the transformation of progesterone to deoxycorticosterone which is the only step occurring in the endoplasmic reticulum. The action could be at the level of intracellular signal transduction for the activation of mitochondrial steroidogenic enzymes or for the transport of steroids in the mitochondria. We showed that guanabenz has an inhibitory effect on the early and late steps of steroidogenesis. This ANF-mimetic effect of guanabenz on mineralocorticoid production despite their widely divergent structures suggests the possibility of some common mechanism of action. They are, however, divergent effects of these two inhibitors. First, in general, the inhibition by guanabenz is more complete than that by ANF. Secondly, accumulation of intermediate products is observed in presence of guanabenz. This latter effect appears to be due to an imbalance between the profound inhibition of the late pathway and the residual activity of the early pathway. These ANF-mimetic effects of guanabenz are also observed with amiloride, a sodium transport blocker which blocks an Na^+/H^+ antiporter in bovine adrenal zona glomerulosa and which interferes with intracellular pH regulation [19]. More investigations have to be done to elucidate and to further compare their mechanism of action. Another interesting point is that the compound guanabenz already has a therapeutic application as an antihypertensive agent. Since guanabenz and amiloride are structurally related with their guanidium moiety and their aromatic ring, it could suggest a new family of antihypertensive agents.

Note added in proof

Since this article was accepted, Lotshaw D. P. *et al.* [*Am. J. Med. Sci.* **301** (1991) 15–20] have shown an inhibition of the early pathway of aldosterone secretion by guanabenz in rat adrenal glomerulosa cells.

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REFERENCES

1. McKenna T. J., Island D. P., Nicholson W. E. and Liddle G. W.: Angiotensin stimulates both early and late steps in aldosterone biosynthesis in isolated bovine glomerulosa cells. *J. Steroid Biochem.* **9** (1978) 967–972.
2. Elliot M. E. and Goodfriend T. L.: Inhibition of aldosterone synthesis by atrial natriuretic factor. *Fedn Proc.* **45** (1986) 2376–2381.
3. Aguilera G., Menard R. H. and Catt K. J.: Regulatory actions of angiotensin II on receptors and steroidogenic enzymes in adrenal glomerulosa cells. *Endocrinology* **107** (1980) 55–60.
4. Kramer R. E., Gallant S. and Brownie A. C.: Actions of angiotensin II on aldosterone biosyntheses in the rat adrenal cortex. Effects on cytochrome *P*-450 enzymes of the early and late pathway. *J. Biol. Chem.* **255** (1980) 3442–3447.
5. Campbell W. B., Currie M. G. and Needleman P.: Inhibition of aldosterone biosynthesis by atriopeptins in rat adrenal cells. *Circ. Res.* **57** (1985) 113–118.
6. Higuchi K., Nawata H., Kato K. I., Ibayashi H. and Matsuo H.: α-human atrial natriuretic polypeptide inhibits steroidogenesis in cultured human adrenal cells. *J. Clin. Endocr. Metab.* **62** (1986) 941–944.
7. Carr B. R. and Mason J. I.: The effects of α-human atrial natriuretic polypeptide on steroidogenesis by fetal zone cells of the human fetal adrenal gland. *Am. J. Obstet. Gynec.* **159** (1988) 1361–1365.
8. Schiebinger R. J., Kem D. C. and Brown R. D.: Effect of atrial natriuretic peptide on ACTH, dibutyryl cAMP, angiotensin II and potassium-stimulated aldosterone secretion by rat adrenal glomerulosa cells. *Life Sci.* **42** (1988) 919–926.
9. Goodfriend T. L., Elliott M. E. and Atlas S. A.: Actions of synthetic atrial natriuretic factor on bovine adrenal glomerulosa. *Life Sci.* **35** (1984) 1675–1682.
10. Racz K., Muchel O., Cantin M. and De Léan A.: Atrial natriuretic factor inhibits the early pathway of steroid biosynthesis in bovine adrenal cortex. *FEBS Lett.* **192** (1985) 19–22.
11. De Léan A., Racz K., Gutkowska J., Nguyen T. T., Cantin M. and Genest J.: Specific receptor-mediated inhibition by synthetic atrial natriuretic factor of hormone-stimulated steroidogenesis in cultured bovine adrenal cells. *Endocrinology* **115** (1984) 1636–1638.
12. Atarashi K., Mulrow P. J. and Franco-Saenz R.: Effect of atrial peptides on aldosterone production. *J. Clin. Invest.* **76** (1985) 1807–1811.
13. De Léan A.: Amiloride potientes atrial natriuretic factor inhibitory action by increasing receptor binding in bovine adrenal zona glomerulosa. *Life Sci.* **39** (1986) 1109–1116.
14. De Léan A., Racz K., McNicoll N. and Desrosiers M. L.: Direct β-adrenergic stimulation of aldosterone secretion in cultured bovine adrenal subcapsular cells. *Endocrinology* **115** (1984) 485–492.
15. Tobes M. C., Hayes S. J., Gildersleeve D. L., Wieland D. M. and Beierwaltes W. H.: Adrenal cortical 11β hydroxylase and side-chain cleavage enzymes. Requirement for the A- or B-pyridyl ring in metyrapone for inhibition. *J. Steroid Biochem.* **22** (1985) 103–110.
16. Brochu M., Féthière J., Roy M., Ong H. and De Léan A.: Highly sensitive and rapid radioimmunoassay for aldosterone in plasma and cell culture medium. *Clin. Biochem.* **22** (1989) 289–292.
17. De Léan A., Munson P. J. and Rodbard D.: Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay and physiological dose–response curves. *Am. J. Physiol.* **235** (1978) E97–E102.
18. Quinn S. J. and Williams H. G.: Regulation of aldosterone secretion. *A. Rev. Physiol.* **50** (1988) 409–426.
19. Horiuchi T., Nguyen T. T., Cragoe E. J. and De Léan A.: Regulation of aldosterone biosynthesis by Na^+/H^+ antiport: relationships between intracellular pH and angiotensin II. *Endocrinology* **124** (1989) 1925–1931.