Actions of Angiotensin II on Aldosterone Biosynthesis in the Rat Adrenal Cortex

EFFECTS ON CYTOCHROME P-450 ENZYMES OF THE EARLY AND LATE PATHWAY

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Previous studies have shown that angiotensin II increases aldosterone biosynthesis, but the mechanisms involved have not been clearly defined. The present studies were carried out to examine the effects of angiotensin II on cytochrome P-450 enzymes of the rat adrenal cortex. Microsomal and mitochondrial cytochrome P-450 levels in the zona glomerulosa were not affected by angiotensin II administration to hypophysectomized rats. Mitochondrial 11β- and 18-hydroxylases of 11-deoxycorticosterone and microsomal 21-hydroxylases of progesterone were also unaffected. In contrast, angiotensin II increased the rate of cholesterol side chain cleavage in glomerulosa mitochondria. The increase in cholesterol side chain cleavage activity correlated with an increase in the heat-generated type I and the pregnenolone-induced type II absorbance changes. In addition, angiotensin II increased the rate of conversion of corticosterone to 18-hydroxycorticosterone and aldosterone, an effect paralleled by an increase in the corticosterone-induced type I absorbance change. Treatment of hypophysectomized rats with cycloheximide had no apparent effect on the action of angiotensin II to promote cholesterol side chain cleavage activity. The effect of angiotensin II to increase the conversion of corticosterone to 18-hydroxycorticosterone and aldosterone, however, was completely abolished by cycloheximide. Activity of cytochrome P-450 enzymes in the zona fasciculata-reticularis was not significantly altered by angiotensin II. The results indicate that angiotensin II promotes aldosterone biosynthesis by increasing both the rate of cholesterol side chain cleavage and the conversion of corticosterone to aldosterone. Increased enzyme activities are mediated, at least in part, by enhanced association of cholesterol and corticosterone with mitochondrial cytochrome P-450, and cytochrome P-450, respectively. The effect of angiotensin II on cholesterol side chain cleavage is apparently independent of protein synthesis. In contrast, its effect on the conversion of corticosterone to aldosterone seems to require the production of a protein factor or factors.

The role of the renin-angiotensin system in the regulation of aldosterone secretion by the adrenal zona glomerulosa has been extensively investigated (1, 2). Most (3–5), but not all (6, 7), studies indicate that angiotensin II and its metabolite angiotensin III ([des-Asp']angiotensin II) increase aldosterone output. The formation of aldosterone from cholesterol by the zona glomerulosa cell involves a series of reactions which include, sequentially, cholesterol → progesterone → pregnenolone → progesterone → 11-deoxycorticosterone → corticosterone → aldosterone (8–10). Most of the enzymes involved in aldosterone biosynthesis have been shown to be NADPH-dependent mixed function oxidases which utilize cytochrome P-450 as their oxygen-activating and substrate-binding component (11–14). Reports by Muller (15) and Kaplan and Bartter (16) and more recently by McKenna et al. (17) and Aguilera and Catt (18) suggest that angiotensin II promotes steroidogenesis by increasing the rate of cholesterol side chain cleavage, i.e., the conversion of cholesterol to pregnenolone. Studies by Haning et al. (19) and Aguilera and associates (18, 20) suggest that angiotensin II also acts to promote the conversion of corticosterone to aldosterone. However, the mechanisms by which angiotensin II increases enzyme activities have not been defined.

Dietary sodium restriction is also a potent stimulus for aldosterone secretion (1, 2). Sodium depletion, like angiotensin II, affects aldosterone biosynthesis by altering enzyme activity at a site in both the early (cholesterol → pregnenolone) (21, 22) and late (corticosterone → aldosterone) (23, 24) biosynthetic pathway. We have recently demonstrated that the increased enzyme activities in response to sodium depletion are accompanied by an increase in the association of cholesterol with cytochrome P-450, and corticosterone with cytochrome P-450, (25). In addition, several recent studies (26–28) have presented evidence that the steroidogenic response of the zona glomerulosa to dietary sodium restriction is mediated, at least in part, by angiotensin II. Thus, the present studies were carried out to examine further the effects of angiotensin II on aldosterone biosynthesis and specifically to determine its effects on cytochrome P-450 enzymes of the zona glomerulosa.

MATERIALS AND METHODS

Animals and Tissue Preparation—Female Sprague-Dawley rats (50 to 55 days old) were obtained from the Holtzman Co., Madison, WI. Animals were singly caged and maintained under standardized conditions of light (0800 to 1800 h) and temperature (22 ± 1°C). Charles River Laboratory Chow containing 0.19 meq of Na+ /g and 0.24 meq of K+/g and tap water were provided ad libitum. Hypophysectomies were performed transaurally, and hormonal treatment was started 24 to 72 h after surgery. Completeness of hypophysectomy was determined by visual inspection at autopsy. Angiotensin II (Isoleucine AII, Sigma) was administered subcutaneously in 0.1 ml of corn oil (50 μg/rat) twice daily for 4 days. When indicated, angiotensin II was given intravenously in 0.1 ml of 0.9% saline (500 μg/rat) 5 min before the animals were killed. Aminogluthethimide (CIBA, 20 mg/ rat) and cycloheximide (Sigma, 10 mg/rat) were administered intraperitoneally in 0.1 ml of 0.9% saline and 0.1 ml of 0.9% saline, 20%
ethanol, respectively, 30 min before the rats were killed. Aminoglutethimide, an inhibitor of the conversion of cholesterol to pregnenolone (29), has been shown to potentiate the effect of ACTH on cholesterol side chain cleavage activity in isolated mitochondria when administered in vivo (30), and was used in the present studies to augment the effect of angiotensin II. Control rats were injected with an equal volume of vehicle only.

Animals were killed by decapitation between 0800 and 1000 h. Adrenal glands were quickly removed and cleaned of adhering fat. The zona glomerulosa and the zona fasciculata-reticularis were separated by incising the capsule and firmly squeezing each adrenal with a needle (31). Adrenal tissue from 40 to 60 rats was pooled and homogenized in 0.25 M sucrose/Tris-HCl (50 mM, pH 7.4) buffer containing 1 mM EDTA to give a single tissue sample per group. Differential centrifugation was used to obtain mitochondria and microsomes (25, 32). Mitochondria were washed twice with sucrose Tris buffer, and the final pellet was resuspended to a concentration of 2 to 4 mg of protein/ml.

Aliquots of the 9,500 × g and the 105,000 × g supernatants were used to assay 21-hydroxylase and cholesterol ester hydrolase activity, respectively. Microsomes were resuspended in 1.15% potassium chloride containing 50 mM Tris-HCl buffer (pH 7.4) to a concentration of 1 to 2 mg/ml and used for spectral studies.

**Enzyme Assays—** Mitochondrial hydroxylase activities were assayed at 37°C as previously described (25). Mitochondria were incubated in Tris-HCl (50 mM, pH 7.4) buffer containing sucrose (50 mM), potassium chloride (5 mM), magnesium chloride (5 mM), sodium chloride (80 mM), EDTA (1 mM), isocitrate (10.1 mM), and 0.5% bovine serum albumin. 11β- and 18-hydroxylase activities were measured as the rates of conversion of exogenous 11-deoxycorticosterone (4.2 μM) to corticosterone and 18-hydroxydeoxycorticosterone, respectively. The conversion of corticosterone to aldosterone and 18-hydroxycorticosterone was assayed by incubating mitochondria with exogenous corticosterone (15 μM). Steroid products were resolved by high pressure liquid chromatography (25, 33) and quantitated by absorbance at 240 nm.

Cholesterol side chain cleavage was assayed as the amount of pregnenolone produced from endogenous cholesterol by mitochondria incubated in the presence of cytochrome P450 (0.01 nm) and a source of reducing equivalents. Pregnenolone was measured by radiomunnoassay (34).

21-Hydroxylase activity was measured as the rate of conversion of exogenous progesterone (42.9 μM) to 11-deoxycorticosterone by 8500 × g supernatant incubated in the presence of sucrose (23.8 mM), magnesium chloride (2.4 mM), potassium chloride (2.4 mM), sodium chloride (38.1 mM), glucose-6-phosphate (4.3 mM), and Tris-HCl (50 mM) buffer (pH 7.4) (25). 11-Deoxycorticosterone was measured by absorbance at 240 nm following resolution by high pressure liquid chromatography (25).

Cholesterol ester hydrolase activity of the 105,000 × g supernatant was assayed by a double isotope method (35, 36) on samples of 0.2 ml (40 to 200 μg of protein) using cholesterol [14C]oleate as substrate. The reaction was allowed to proceed for 15 min at 37°C (pH 7.4). The [14C]Oleate product was extracted (37) and counted in a Packard Tri-Carb liquid scintillation spectrometer.

**Substrate-induced Absorbance Changes—** Substrate-induced absorbance changes were measured using an Aminco DW-2 recording spectrophotometer. Heat-generated type I absorbance changes, indicative of cholesterol association with cytochrome P450c, were recorded while warming the mitochondria from 0°C to 37°C (38). The change in the temperature-induced spectrum as the mitochondria warmed from approximately 10°C to 25°C is correlated with the rate of cholesterol side chain cleavage (39) and was used as an index of the rate of association of cholesterol with cytochrome P450c. Pregnenolone-induced type II absorbance changes, indicative of cholesterol displacement from cytochrome P450c, and 11-deoxycorticosterone-induced type I absorbance changes were measured at 37°C. Corticosterone-induced type I absorbance changes were measured at 0°C. Microsomal progesterone-induced type I absorbance changes were measured at 37°C. Mitochondrial and microsomal cytochrome P450c were measured as the dithionite-reduced carbon monoxide complex using a millimolar extinction coefficient of 91 (40). NADPH-cytochrome c reductase activity was assayed as described by Phillips and Langdon (41). Adrenal mitochondrial and microsomal protein were measured by the method of Lowry et al. (42).

**Serum Corticosterone Assays—** Serum aldosterone was measured by radioimmunoassay following chromatography in a system of benzene to cyclohexane to methanol to water (85:15:50:25, v/v/v) (43). Corticosterone was measured by radioimmunoassay after methylene chloride extraction but without chromatography (44).

**RESULTS**

**Effects of Angiotensin II on Adrenal Weight and Peripheral Corticosteroid Levels—** Administration of angiotensin II (100 μg/rat/day) to hypophysectomized female rats for 4 days had no apparent effect on body and adrenal weights (Table 1). As expected, angiotensin II treatment increased the peripheral serum level of aldosterone (Table 1). The serum level of corticosterone was also increased by angiotensin II (Table 1).

Acute treatment of hypophysectomized rats with 500 μg of angiotensin II had similar, but more marked, effects on serum levels of both corticosterone and aldosterone (Fig. 1).

**Effects of Angiotensin II on Cytochrome P-450 Enzymes of the Zona Glomerulosa—** Cholesterol side chain cleavage activity was assayed in glomerulosa mitochondria isolated from hypophysectomized female rats that had been given angiotensin II subcutaneously (100 μg/rat/day) for 4 days and then intravenously (500 μg/rat) 5 min before being killed. Angiotensin II had no demonstrable effect on the rate of cholesterol side chain cleavage when given to hypophysectomized rats (Table II). In contrast, when given to hypophysectomized rats that had been treated with aminoglutethimide 30 min before being killed, angiotensin II significantly increased the rate of cholesterol side chain cleavage in glomerulosa mitochondria.

**TABLE I**

| Angiotensin II (All, 50 μg/rat) was administered to hypophysectomized (HYPOX) female rats twice daily for 4 days. On the 5th day, animals were killed approximately 2 h after angiotensin II administration. There were 12 animals per group.|
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Body weight (g)** | **HYPOX** | **HYPOX + All** |
| 1.0 | 165.9 ± 3.8 | 163.1 ± 3.7 |
| 2.0 | 25.5 ± 0.5 | 25.9 ± 1.0 |
| 3.0 | 0.31 ± 0.05 | 0.56 ± 0.10 |
| 4.0 | 22.1 ± 3.0 | 66.3 ± 4.4 |

* Values are expressed as mean ± S.E. *p < 0.01 (versus hypox group).

**Fig. 1.** Effects of acute angiotensin II treatment on peripheral serum levels of corticosterone (○) and aldosterone (○). Groups of 10 hypophysectomized female rats were killed 5, 10, and 15 min after the intravenous administration of angiotensin II (500 μg/rat). Control animals (time 0) received an equal volume (0.1 ml) of vehicle (0.9% saline) only and were killed immediately. Symbols indicate the mean of 10 determinations: *p < 0.001 (versus corticosterone level at time 0). †p < 0.001 (versus aldosterone level at time 0). The vertical bars represent ± S.E.
Effects of angiotensin II on cholesterol side chain cleavage activity of zona glomerulosa mitochondria

Hypophysectomized (HYPOX) female rats were treated with angiotensin II (All, 50 μg/rat) twice daily for 4 days. On Day 5, All (500 μg/rat) was administered intravenously 5 min before the rats were killed. In the first experiment All was given to HYPOX rats that had been pretreated for 25 min with aminoglutethimide (AG, 20 mg/rat). In a third experiment All was given to HYPOX rats pretreated for 25 min with AG plus cycloheximide (CH, 10 mg/rat). Each experiment was performed three times.

<table>
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<th>Side chain cleavage</th>
<th>HYPOX</th>
<th>HYPOX + AG</th>
<th>HYPOX + AG + CH</th>
<th>HYPOX + CH</th>
<th>HYPOX + AG + CH</th>
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<td>(nmol/min/mmol P-450)</td>
<td>0.91 ± 0.11</td>
<td>1.00 ± 0.06</td>
<td>0.81 ± 0.09</td>
<td>1.89 ± 0.18</td>
<td>0.93 ± 0.01</td>
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<td>HGI (ΔA420-450)</td>
<td>50.5 ± 4.7</td>
<td>51.5 ± 4.0</td>
<td>38.9 ± 8.0</td>
<td>71.0 ± 3.4</td>
<td>33.7 ± 2.6</td>
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<tr>
<td>HGI (10^3 × ΔA/min/nmol P-450)</td>
<td>6.2 ± 0.4</td>
<td>6.5 ± 0.2</td>
<td>7.3 ± 0.8</td>
<td>17.0 ± 1.7</td>
<td>10.6 ± 0.1</td>
</tr>
<tr>
<td>PII (ΔA430-450)</td>
<td>48.8 ± 4.0</td>
<td>49.5 ± 3.9</td>
<td>34.7 ± 2.6</td>
<td>65.5 ± 2.4</td>
<td>32.8 ± 2.2</td>
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</tbody>
</table>

* Values are expressed as the mean ± S.E. of three experiments.
<sup>p</sup> <i>p < 0.01 (versus the control group of the same experiment).
<sup>'p</sup> <i>p < 0.05 (versus the control group of the same experiment).

The increase in the rate of cholesterol side chain cleavage was accompanied by an increase in the rate and the magnitude of the heat-generated type I absorbance change (Table II). The magnitude of the pregnenolone-induced type II absorbance change was also increased (Table II). The increases in the rate of cholesterol side chain cleavage and the association of cholesterol with cytochrome P-450<sub>24</sub> were demonstrable even after acute angiotensin II treatment (Table III). Acute angiotensin II treatment had no apparent effect on soluble cholesterol ester hydrolase and mitochondrial NADPH-cytochrome c reductase activities (Table III). The activities of other adrenocortical cytochrome P-450 enzymes were not affected by the acute administration of angiotensin II or aminoglutethimide.

The rate of conversion of corticosterone to 18-hydroxycorticosterone and aldosterone by glomerulosa mitochondria was increased by chronic angiotensin II treatment (Table IV). In addition, angiotensin II increased the magnitude of the corticosterone-induced type I absorbance change (Table IV), indicating enhanced association of corticosterone with cytochrome P-450<sub>24</sub>. Preliminary results suggest that angiotensin II also increases the affinity of cytochrome P-450<sub>24</sub> for corticosterone, as indicated by the corticosterone spectral dissociation constant (K<sub>c</sub>) (Table IV).

An increase in the rate of conversion of corticosterone to 18-hydroxycorticosterone and aldosterone by glomerulosa mitochondria was demonstrable 3 h after a single injection of angiotensin II (Fig. 2). Maximal enzyme activity was obtained only after several days of treatment (Fig. 2).

The rates of 11β- and 18-hydroxylation of 11-deoxycorticosterone by glomerulosa mitochondria were not affected by angiotensin II treatment (Table V).<sup>1</sup> The magnitude of the 11-deoxycorticosterone-induced type I absorbance change was also unaffected (Table V). Similarly, microsomal 21-hydroxylase activity and the magnitude of the progesterone-induced type I absorbance change in the zona glomerulosa were not significantly affected by angiotensin II (Table VI). Angiotensin II had no apparent effect on glomerulosa microsomal (Table VI) and mitochondrial (Table V) protein and cytochrome P-450 concentrations.

Effects of Angiotensin II on Cytochrome P-450 Enzymes of the Zona Fasciculata-Reticularis—In contrast to its actions in the zona glomerulosa, angiotensin II had no apparent effects on the activities of or substrate interaction with cytochrome P-450 enzymes in the inner zones of the rat adrenal cortex (Tables V to VII).

Effects of Cycloheximide on the Actions of Angiotensin II—In order to compare the effects of cycloheximide on the actions of angiotensin II on cholesterol side chain cleavage and the conversion of corticosterone to 18-hydroxycorticosterone and aldosterone within the zona glomerulosa, hypophysectomized rats were treated for 4 days with angiotensin II. On the 5th day aminogluthethimide and cycloheximide were given 30 min before and angiotensin II 5 min before the rats were killed. Cycloheximide had no apparent effect on the actions of angiotensin II to promote the association of cholesterol with cytochrome P-450<sub>24</sub> and to enhance the rate of cholesterol side chain cleavage (Tables II and III). In contrast, cycloheximide completely abolished the effect of angiotensin II to increase the rate of conversion of corticosterone to 18-hydroxycorticosterone and aldosterone (Table IV). The addition of cycloheximide to isolated zona glomerulosa mitochondria <i>in vitro</i> did not significantly affect the rate of formation of aldosterone and 18-hydroxycorticosterone from cor-

<sup>1</sup> Tables V to VIII are available in miniprint format at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9560 Rockville Pike, Bethesda, Md. 20014. Request Document No. 70M-1011, cite author(s), and include a check or money order for $1.00 per set of photocopies.
Angiotensin II and Aldosterone Biosynthesis

Effects of angiotensin II on the conversion of corticosterone (B) to 18-hydroxy corticosterone (18-OH-B) and aldosterone by zona glomerulosa mitochondria

Refer to Table II for details.

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<th></th>
<th>HYPOX</th>
<th>HYPOX + All</th>
<th>HYPOX + AG</th>
<th>HYPOX + AG + All</th>
<th>HYPOX + AG + CH</th>
<th>HYPOX + AG + CH + All</th>
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<tr>
<td>B 18-OH-B</td>
<td>11.2 ± 0.7</td>
<td>26.1 ± 1.1</td>
<td>9.6 ± 0.6</td>
<td>18.7 ± 1.5</td>
<td>9.3 ± 1.5</td>
<td>9.7 ± 1.3</td>
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<tr>
<td>B aldosterone</td>
<td>2.8 ± 0.3</td>
<td>6.7 ± 0.2</td>
<td>3.3 ± 0.2</td>
<td>6.8 ± 0.6</td>
<td>3.1 ± 0.6</td>
<td>3.2 ± 0.5</td>
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<tr>
<td>B1 ΔA(4,20-Δ)</td>
<td>14.4 ± 1.4</td>
<td>25.2 ± 1.8</td>
<td>ND</td>
<td>ND</td>
<td>9.4</td>
<td>10.8</td>
</tr>
<tr>
<td>B K(10^-5 M)</td>
<td>12.5</td>
<td>6.2</td>
<td>ND</td>
<td>ND</td>
<td>9.0</td>
<td>5.2</td>
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Values are expressed as the mean ± S.E. of three experiments.

*p < 0.01 (versus the control group of the same experiment).

Not determined.

Single determination.

![Graph](image)

**Fig. 2.** Effects of angiotensin II (All) on the conversion of corticosterone (B) to aldosterone and 18-hydroxycorticosterone (18-OH-B) by zona glomerulosa mitochondria. Hypophysectomized female rats were treated with All for 3, 12, 48, 96, and 120 h. Rats killed after 3 and 12 h of treatment were given a single subcutaneous injection of 50 μg of All in 0.1 ml of corn oil at time 0. Rats killed after 48 h of treatment were given 50 μg of All at 12-h intervals. Mitochondria were isolated from the zona glomerulosa and the conversion of corticosterone to aldosterone (C) and 18-OH-B (●) was measured as described under “Materials and Methods.”

Corticosterone (Table VIII). The magnitude of the corticosterone-induced type I absorbance change was also similar in the presence and absence of cycloheximide (Table VIII).

**DISCUSSION**

Previous studies have suggested that angiotensin II has a dual effect on the steroidogenic pathway to promote aldosterone output. Muller (15) and Kaplan and Barter (16) first reported that the addition of angiotensin II to adrenal tissue in vitro increased the conversion of isotopic cholesterol to pregnenolone. More recent studies by Aquilera and Catt (18) and McKenna et al. (17) provide additional support for an action of angiotensin II prior to the formation of pregnenolone. The data presented in this communication confirm an effect of angiotensin II early in the aldosterone biosynthetic pathway and demonstrate that it acts to increase the rate of cholesterol side chain cleavage, the conversion of cholesterol to pregnenolone. Increased cholesterol side chain cleavage activity appears to be important in the acute stimulation of aldosterone biosynthesis and results primarily in an increase in precursor (corticosterone) formation. Angiotensin II also has an effect on the aldosterone biosynthetic pathway at a site after the formation of pregnenolone; i.e. at the last step in aldosterone biosynthesis, the conversion of corticosterone to aldosterone. The conversion of corticosterone to aldosterone appears to be the rate-limiting step in aldosterone biosynthesis, and its regulation becomes important in the long term control of aldosterone production. Although an increase in the rate of conversion of corticosterone to 18-hydroxycorticosterone and aldosterone is demonstrable within several hours of angiotensin II administration, maximal enzyme activity is not achieved until several days of treatment. Thus, in contrast to the early pathway (cholesterol side chain cleavage), the late pathway (conversion of corticosterone to aldosterone) requires more chronic exposure to angiotensin II before it contributes to an increase in aldosterone biosynthesis. Komor and Muller (45) have also reported that administration of angiotensin II promotes the conversion of corticosterone to aldosterone. They, however, noted that the rate of aldosterone production from exogenous corticosterone gradually returned to control levels despite continued angiotensin II administration. The difference in the duration of angiotensin II action on the late pathway reported here and by Komor and Muller might reflect the presence and absence of the pituitary gland; therefore, the presence and absence of ACTH and perhaps other factors that might directly affect enzyme activity modify the response to angiotensin II, or both. In addition, an action of angiotensin II on cholesterol side chain cleavage activity within the zona glomerulosa as well as the presence of a functional zona fasciculata-reticularis might have contributed to the apparent decline in the conversion of isotopic corticosterone to aldosterone reported by Komor and Muller. Regardless, our results demonstrate that angiotensin II has a direct and independent action to increase the rate of conversion of corticosterone to 18-hydroxy corticosterone and aldosterone.

It is generally accepted that corticosterone is the immediate precursor for aldosterone. However, several recent studies (17, 18, 46, 47) have suggested the presence of an additional late pathway within the zona glomerulosa cell that would preferentially utilize 11-deoxycorticosterone for aldosterone biosynthesis. Presumably, 11-deoxycorticosterone is converted to 18-hydroxydeoxycorticosterone, 18-hydroxycorticosterone, and subsequently to aldosterone. Yet, the importance of such a pathway in the response of the zona glomerulosa to angiotensin II remains unclear. McKenna et al. (17) reported that angiotensin II increased the rate at which glomerulosa cells converted 11-deoxycorticosterone, but not corticosterone, to aldosterone. In contrast, Aquilera and Catt (18) and Haning et al. (19) noted that the rate of conversion of corticosterone to aldosterone by glomerulosa cells was enhanced by angiotensin II. The rate of conversion of 11-deoxycorticosterone to aldosterone, on the other hand, was not affected (18). In
addition, angiotensin II had no apparent effect on the metabolism of 11-deoxycorticosterone by isolated glomerulosa mitochondria in the present studies. Although we cannot exclude the possibility that angiotensin II enhances the conversion of 18-hydroxydeoxycorticosterone to aldosterone, our studies suggest that the action of angiotensin II on the late steroidogenic pathway, i.e., after the formation of pregnenolone, is restricted to the conversion of corticosterone to aldosterone and 18-hydroxydeoxycorticosterone.

The actions of angiotensin II to increase the conversion of cholesterol to pregnenolone and corticosterone to aldosterone seems to be mediated by an effect on the cytochrome P-450 enzymes involved. For example, the increase in cholesterol side chain cleavage activity was paralleled by increases in the heat-generated type I and the pregnenolone-induced type II absorbance changes. In addition, an increase in the corticosterone-induced type I absorbance change accompanied the increased rate of conversion of corticosterone to 18-hydroxydeoxycorticosterone and aldosterone. These observations suggest that angiotensin II enhances the rate of cholesterol side chain cleavage and the conversion of corticosterone to aldosterone by promoting the association of cholesterol with cytochrome P-450, and corticosterone with cytochrome P-450, respectively.

The effect of angiotensin II on cholesterol side chain cleavage in the zona glomerulosa reported here is similar to that reported for ACTH in both the zona glomerulosa and the zona fasciculata-reticularis (8). The effect of ACTH seems to require activation or production of a labile protein (49), and it has been proposed that this protein acts within the mitochondrion to facilitate the association of cholesterol with cytochrome P-450, (50, 51). Inhibitors of protein synthesis such as puromycin and cycloheximide have also been shown to block the steroidogenic response of the adrenal to angiotensin II (52, 53). In addition, our data indicate that angiotensin II, like ACTH, acts to promote the interaction of cholesterol with cytochrome P-450, in zona glomerulosa mitochondria. Nonetheless, the effects of angiotensin II on cholesterol-cytochrome P-450, interaction and cholesterol side chain cleavage activity were not prevented by cycloheximide. Thus, the mechanism by which angiotensin II enhances cholesterol association with cytochrome P-450, increases the rate of cholesterol side chain cleavage seems to differ from that of ACTH. Recent studies (54-56) comparing the effects of angiotensin II and ACTH on cyclic nucleotide generation in the adrenal cortex also suggest a divergence in the mechanisms by which angiotensin II and ACTH promote steroidogenesis.

It is possible that angiotensin II modifies cholesterol side chain cleavage activity by influencing the distribution of cholesterol within the zona glomerulosa cell. For example, uptake of free cholesterol from plasma (57), hydrolysis of cholesterol esters (58, 59), and uptake of free cholesterol by mitochondria (60) have been implicated in the effect of ACTH on steroidogenesis. Similarly, angiotensin II could influence steroid output by affecting the availability of cholesterol. However, our preliminary results suggest that angiotensin II has no appreciable effect on the hydrolysis of cholesterol esters. Brecher et al. (60) noted that angiotensin II also failed to affect the rate of cholesterol esterification. Nonetheless, an action of angiotensin II to increase the level of free cholesterol within the mitochondrion cannot be excluded. In fact, Aguileria and Catt (28) have reported that mitochondrial cholesterol levels within the zona glomerulosa are elevated after chronic sodium depletion, an effect apparently mediated by angiotensin II. It is presently unknown whether the increase in the rate of cholesterol side chain cleavage observed in response to acute angiotensin II treatment is accompanied by an increase in the uptake of cholesterol by or a redistribution of cholesterol within the mitochondrion.

The effect of angiotensin II on the conversion of corticosterone to aldosterone, in contrast to its effect on cholesterol side chain cleavage activity, seems to require de novo protein synthesis. For example, cycloheximide treatment in vitro completely blocked the action of angiotensin II to increase the rate of conversion of corticosterone to aldosterone and 18-hydroxydeoxycorticosterone. The increase in the corticosterone-induced type I absorbance change produced by angiotensin II was also prevented by cycloheximide. Puromycin has been shown to inhibit the conversion of corticosterone to aldosterone when added to glomerulosa mitochondria in vitro (61, 62). Lehoux and Forest (62) have also reported that puromycin interacts with mitochondrial cytochrome P-450 and that cycloheximide decreases the magnitude of the puromycin-induced absorbance change. These observations raise the possibility that the effects of cycloheximide in vitro observed in the present studies reflect a direct inhibitory action at the level of the mitochondrion. Our observations, however, suggest that cycloheximide does not directly inhibit the interaction of corticosterone with cytochrome P-450, and cycloheximide does not directly inhibit the interaction of corticosterone with cytochrome P-450, in vitro did not affect the rate of conversion of corticosterone to 18-hydroxydeoxycorticosterone and aldosterone. Thus, excluding an action on some other parameter within the zona glomerulosa cell, the effect of cycloheximide in vitro appears to be due to an inhibition of protein synthesis. Angiotensin II apparently induces the de novo synthesis of a protein that promotes the association of corticosterone with cytochrome P-450, and increases corticosterone 18-hydroxylase activity.

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**Table I**

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Supplement to

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<th>0.1</th>
</tr>
</thead>
</table>

**Table VII**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>0.0</th>
<th>0.0</th>
<th>0.0</th>
</tr>
</thead>
</table>

**Table VIII**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>0.0</th>
<th>0.0</th>
<th>0.0</th>
</tr>
</thead>
</table>

**Table IX**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>0.0</th>
<th>0.0</th>
<th>0.0</th>
</tr>
</thead>
</table>