Effect of Angiotensin II on Renin Production by Rat Adrenal Glomerulosa Cells in Culture

Tatsuyuki Yamaguchi, Roberto Franco-Saenz, and Patrick J. Mulrow

Angiotensin II (Ang II) inhibits renin secretion and production from the kidney, but the effect of Ang II on adrenal renin is not clear. Nephrectomy, via elevated plasma adrenocorticotropic hormone (ACTH) and potassium, is a strong stimulator of adrenal renin production in the rat. This stimulation is inhibited by the infusion of Ang II, suggesting a negative feedback between Ang II and adrenal renin. In the present study, we examined the effect of Ang II on adrenal renin using a primary culture of rat glomerulosa cells. Cells were exposed to ACTH (10^{-11} M), high potassium (8 and 12 mM), db-cyclic AMP (db-cAMP) (10^{-3} M), or Ang II (10^{-11} to 10^{-5} M) for 24 hours, and active renin and inactive renin were measured. Active renin was predominant in the cells, whereas inactive renin predominated in the medium. Ang II stimulated renin production in a dose-dependent fashion (cell-active renin, 1.21±0.20 to 2.39±0.16; medium-inactive renin, 2.59±0.40 to 6.14±0.49 ng Ang I/10^6 cells). Both ACTH and db-cAMP significantly stimulated active renin in the cells (ACTH, 1.73±0.14 to 9.44±0.98; db-cAMP, 1.45±0.16 to 3.96±0.71 ng Ang I/10^6 cells) and inactive renin in the medium (ACTH, 4.98±0.38 to 43.7±5.63; db-cAMP, 3.80±0.32 to 33.5±5.62 ng Ang I/10^6 cells). The addition of Ang II (10^{-7} M) blunted the stimulation of renin production by both ACTH and db-cAMP by 60%. High potassium-stimulated renin production was not inhibited by Ang II. In conclusion, basal renin production is increased by Ang II, but Ang II inhibits ACTH-stimulated adrenal renin production. This effect is due to blockade of the action of cAMP on renin production.

**Key Words** • renin-angiotensin system • cell cultures • angiotensin II • adrenocorticotropic hormone • potassium • cyclic AMP

The existence of the renin-angiotensin system in the adrenal gland is now generally accepted, and the factors that regulate adrenal renin have been examined by several investigators. Our laboratory reported that adrenal renin is stimulated by low sodium and high potassium diet and by bilateral nephrectomy in the rat in vivo. Although circulating angiotensin II (Ang II) is an important factor in the kidney that inhibits the secretion of renin and production of renin, the effect of Ang II on adrenal renin has not been clear.

We reported that after bilateral nephrectomy, the stimulation of adrenal renin, which is due to the increased levels of plasma potassium and adrenocorticotropin hormone (ACTH), is inhibited by the infusion of Ang II. This finding suggests the existence of a negative feedback system between circulating Ang II and adrenal renin, similar to the kidney. Recently, we developed a primary monolayer culture of adrenal glomerulosa cells of the rat and demonstrated increased production of adrenal renin and aldosterone in response to ACTH and high potassium. The purpose of this study was to characterize the effect of Ang II on renin production in the adrenal gland using the primary monolayer culture of adrenal glomerulosa cells of the rat.

**Methods**

**Cell Culture**

Female Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, Ind.) weighing 150–175 g were killed by decapitation, the adrenals were removed, and the capsular portion (zona glomerulosa cells) was separated from the decapsular portion (fasciculata medullary cells). The capsular portion was cut into small fragments, and the cells were dispersed by collagenase and suspended in PFMFR-4 medium (Biofluid Inc., Rockville, Md.) with 10% fetal calf serum with the additives described previously. The cell suspensions were seeded in 24-well plastic culture plates (Corning, N.Y.), coated with fibronectin (0.7–1.0×10^5 cells per well), and incubated in an atmosphere of 95.5% air and 4.5% CO_2 at 37°C. After 48 hours of incubation to allow for cell attachment, the medium containing fetal calf serum was removed from the wells, the cells were washed twice with Medium-199 (Sigma Chemical Co., St. Louis, Mo.) and were supplemented with vitamin C (18 μg/ml), vitamin E (0.47 μg/ml), penicillin G (100 units/ml), streptomycin (100 μg/ml), fungizone (1 μg/ml), and 0.2% bovine serum albumin. Approximately 70% of initially seeded cells remained attached to the wells.

Then the supplemented Medium-199 (K^+, 3.5 mM) was replenished, and the cells were exposed to ACTH (10^{-11} M, fragment 1–24, Sigma), high potassium (8 and 12 mM), N^6-O^2'- dibutyryl adenosine 3':5' - cyclic monophos-
Renin inhibitor also inhibited these renin activities by 90% at a concentration of 100 nM. 

Radioimmunoassay of Renin and Aldosterone

Cells stored at -70°C were thawed at 4°C and frozen again on dry ice. The freezing/thawing cycle was repeated three times to homogenize the cells before the assay. As described previously,11 this process does not alter the ratio of active to inactive renin in the cells because when adrenal capsules are quickly homogenized and renin is measured, the ratio is very similar to what is found in the cells after the freezing and thawing process. Active renin in the cell homogenate was measured as previously described.11 Total renin was measured by the trypsin activation method.11 The cell homogenate was incubated with trypsin (0.1 mg/ml sample, type II-L, Sigma). Inactive renin was calculated as the difference between total renin and active renin. Renin activity in the medium and in the cells was measured by the trypsin activation method.11,12 The cell homogenate was incubated with trypsin (0.1 mg/ml sample, type III, Sigma) for 1 hour at 4°C. The reaction was stopped by the addition of lima bean trypsin inhibitor (0.3 mg/ml sample, type II-L, Sigma). Inactive renin was calculated as the difference between total renin and active renin. Renin activity in the medium was measured by the same method as cellular renin activity, except 200 µl medium (pH 7.4) was used as a sample. As described previously,11 both active renin and trypsin-activated inactive renin are inhibited by an anti-hog kidney renin monoclonal antibody13 and the renin inhibitor (CP71362, Pfizer Laboratories, New York City). This renin inhibitor completely inhibited renin activity in a rat kidney extract at 1 µM, and 100 nM caused an 80% inhibition of the kidney renin activity. Approximately 80% of active and total renin activity both in the cells and in the medium was inhibited by 1:100 dilution of the antibody, and the renin inhibitor also inhibited these renin activities by 90% at a concentration of 100 nM.

Aldosterone in the medium was measured by direct assay using a radioimmunoassay kit (Coat-a-Count, Diagnostic Products, Los Angeles).

Statistical Analysis

Results are normalized to one million cells for renin (ng angiotensin I [Ang I]/one million cells/8 hours of renin assay incubation) and for aldosterone (ng one million cells). The results of the experiments were expressed as the mean±SEM. The data were analyzed statistically by one-factor analysis of variance and Fisher's probability of the least significant difference test. Significance was defined as a value of p<0.05.

Results

Figure 1 shows the effect of Ang II on aldosterone secretion. Ang II stimulated aldosterone secretion into the medium in a dose-dependent fashion (from 84.0±14.2 in control to 335.2±67.0 ng/10^6 cells at 10^{-4} M).

Figure 2 shows the effect of Ang II on renin activity in the medium and in the cells. In the medium, inactive renin accounted for a dominant part of total renin activity, whereas in the cells, active renin was dominant. Ang II stimulated inactive renin in the medium from 2.59±0.40 in control to 6.14±0.49 ng Ang I/10^6 cells/8 hr at 10^{-5} M, whereas active renin in the medium was not stimulated. In the cells, active renin was stimulated by Ang II; active renin increased from 1.21±0.20 to 2.39±0.16 ng Ang I/10^6 cells/8 hr at 10^{-5} M.

To verify if these stimulatory effects of Ang II were mediated by the specific receptor for Ang II, the effect of the nonpeptide Ang II antagonist DuP 753 (Du Pont Company, Wilmington, Del.) was examined. Various amounts of DuP 753 (10^{-8} to 10^{-3} M) were added to the culture medium containing 10^{-7} M of Ang II and incubated for 24 hours. DuP 753 inhibited Ang II-stimulated aldosterone, inactive renin secretion in the medium, and active renin in the cells dose dependently and caused almost complete inhibition at 10^{-5} M (Figure 3); however, DuP 753 had no effect on the basal levels of aldosterone, renin in the cells, and renin in the medium (data not shown).

Figure 4 shows the effect of Ang II on ACTH-stimulated renin in the medium and in the cells. In this experiment, 10^{-11} M ACTH was added to the medium. This concentration of ACTH has been shown to cause 50% maximal stimulation of renin production.11 In the medium, 10^{-11} M ACTH alone stimulated inactive renin significantly, and the addition of Ang II (10^{-7} M) inhibited this stimulation (control, 4.98±0.38; ACTH, 43.7±5.63; ACTH/Ang II, 19.78±1.31 ng Ang I/10^6.
FIGURE 2. Bar graphs show effect of angiotensin II on renin activity in the medium and in the cells. Each bar represents mean ± SEM. Renin activity is expressed as nanograms angiotensin I per one million cells per 8 hours of renin assay incubation. n, Number of culture wells assayed in three separate experiments. *p<0.05, **p<0.01 compared with active renin in control. +p<0.05, ++p<0.01 compared with inactive renin in control. AI, angiotensin I; A II, angiotensin II.

To further study the intracellular mechanism of ACTH stimulation, the effect of db-cAMP (10⁻³ M) was examined. Figure 5 shows the effect of db-cAMP and the combination of db-cAMP and Ang II on renin activity in the medium and in the cells. Inactive renin in the medium was stimulated significantly by db-cAMP, and the addition of Ang II (10⁻⁷ M) inhibited this stimulation (control, 3.80±0.32; db-cAMP, 33.55±5.62; db-cAMP/Ang II, 15.49±0.47 ng Ang I/10⁶ cells/8 hr). Active renin in the medium was also stimulated by db-cAMP with no significant inhibitory effect by Ang II (control, 0.48±0.16; db-cAMP, 1.19±0.25; db-cAMP/Ang II, 1.35±0.22 ng Ang I/10⁶ cells/8 hr). In the cells, db-cAMP did not stimulate inactive renin significantly.

FIGURE 3. Line graph shows effect of nonpeptide angiotensin II receptor antagonist (DuP 753) on angiotensin II–stimulated aldosterone and renin activity from one experiment run in duplicate. From 10⁻⁸ to 10⁻⁴ M of DuP 753 was added to the medium containing 10⁻⁷ M angiotensin II. After 24 hours incubation, aldosterone in the medium and renin in the medium and in the cells were measured. Response to angiotensin II alone is considered as 100%. Aldosterone, aldosterone in the medium; medium inactive, inactive renin in the medium; cell active, active renin in the cells. Ang II, angiotensin II.
Figure 4. Bar graphs show effect of angiotensin II ($10^{-7}$ M) on adrenocorticotropic hormone (ACTH) ($10^{-11}$ M)-stimulated renin activity in the medium and in the cells. Each bar represents mean±SEM. Logarithmic axis is used for the vertical axis to avoid invisible bars for low levels of medium active renin. n, Number of culture wells assayed in three separate experiments. *p<0.05, **p<0.01 compared with active renin in control. +p<0.05, ++p<0.01 compared with inactive renin in control. AI, angiotensin I; A II, angiotensin II.

(control, 0.24±0.11; db-cAMP, 0.83±0.28; db-cAMP/Ang II, 0.26±0.16 ng Ang I/10⁶ cells/8 hr); however, active renin was significantly stimulated and the stimulation was blunted by Ang II (control, 1.47±0.16; db-cAMP, 3.96±0.41; db-cAMP/Ang II, 2.31±0.15 ng Ang I/10⁶ cells/8 hr).

Figure 6 shows the effect of Ang II on high potassium-stimulated renin activity. Inactive renin in the medium and both active and inactive renin in the cells were stimulated by high potassium in a dose-dependent fashion. The stimulated renin levels after high potassium, even at 12 mM, were lower than those after ACTH or db-cAMP stimulation (approximately one fourth and one third compared with ACTH and db-cAMP, respectively). Medium inactive renin increased from 5.14±0.07 in control to 11.27±0.87 after 12 mM K⁺; active renin in the cells from 1.02±0.10 to 2.06±0.10; inactive renin in the cells from 0.17±0.15 to 1.43±0.27 ng Ang I/10⁶ cells/8 hr. The addition of Ang II to the high potassium medium did not inhibit the high potassium-stimulated renin activity but had an additive effect (medium inactive renin, 16.00±0.36; cell active renin, 2.92±0.39; cell inactive renin, 2.47±0.04 after the addition of Ang II at 12 mM K⁺).

Table 1 shows the effect of Ang II on ACTH-, db-cAMP-, and high potassium-stimulated aldosterone secretion in the medium. ACTH, db-cAMP, and high potassium-stimulated aldosterone secretion by 19-fold, 13-fold, and ninefold, respectively. The addition of Ang II showed no significant effect on ACTH- and db-cAMP-stimulated aldosterone, although there was slight increase. High potassium-stimulated aldosterone further increased after the addition of Ang II.

Discussion

The effect of Ang II on adrenal renin is complex and different from that on the kidney. It has both a stimulatory and an inhibitory effect on adrenal renin production by the cultured adrenal glomerulosa cells in the rat. Ang II alone had comparatively weak but significant stimulatory effect on the levels of active renin in the cells and inactive renin in the medium. Because inactive renin is regarded as prorenin and likely to be secreted constitutively, the predominant increase in inactive renin in the medium may reflect the activation of renin messenger RNA (mRNA) transcript in the cells and subsequent increase in prorenin and renin production. In fact, the increase in active renin in the cells after any stimulation is always associated with an increase in inactive renin secretion into the medium, and there is a strong positive correlation between the two ($r=0.85$, $p<0.001$). Therefore Ang II probably stimulates renin synthesis in the glomerulosa cells. The stimulatory effect of Ang II on renin and aldosterone was completely inhibited by DuP 753, which is a specific nonpeptide angiotensin subtype 1 (AT₁) receptor antagonist, indicating that the stimulatory effect of Ang
Yamaguchi et al  Effect of Angiotensin II on Adrenal Renin  

**TABLE 1.** Effect of Angiotensin II on Stimulated Aldosterone Secretion

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Aldosterone (ng/10^6 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>64.0±4.1</td>
</tr>
<tr>
<td>Ang II</td>
<td>374.7±15.0*</td>
</tr>
<tr>
<td>ACTH</td>
<td>1,232.9±65.9*</td>
</tr>
<tr>
<td>ACTH/Ang II</td>
<td>1,367.6±77.7*</td>
</tr>
<tr>
<td>Control</td>
<td>59.4±10.1</td>
</tr>
<tr>
<td>db-cAMP</td>
<td>781.6±19.0*</td>
</tr>
<tr>
<td>db-cAMP/Ang II</td>
<td>813.3±45.0*</td>
</tr>
<tr>
<td>Control</td>
<td>107.7±5.0</td>
</tr>
<tr>
<td>Ang II</td>
<td>286.0±18.7*</td>
</tr>
<tr>
<td>K 8 mM</td>
<td>221.7±23.3†</td>
</tr>
<tr>
<td>K 8 mM/Ang II</td>
<td>572.8±49.8*</td>
</tr>
<tr>
<td>K 12 mM</td>
<td>947.4±48.1*</td>
</tr>
<tr>
<td>K 12 mM/Ang II</td>
<td>1,178.1±40.7*</td>
</tr>
</tbody>
</table>

Effect of angiotensin II (Ang II) on adrenocorticotropic hormone (ACTH) (10^{-9} M), N^6-O^4-dibutyryladenosine 3':5'-cyclic monophosphate (db-cAMP) (10^{-3} M), or high potassium (8 and 12 mM)-stimulated aldosterone in culture medium. Data represent mean±SEM. NS, no significant difference.

**FIGURE 5.** Bar graphs show effect of angiotensin II (10^{-7} M) on N^6-O^4-dibutyryladenosine 3':5'-cyclic monophosphate (db-cAMP) (10^{-3} M)-stimulated renin activity in the medium and in the cells. Each bar represents mean±SEM. n, Number of culture wells assayed in three separate experiments. *p<0.05, **p<0.01 compared with active renin in control. +p<0.05, ++p<0.01 compared with inactive renin in control. AI, angiotensin I; A II, angiotensin II.
Hypertension Vol 19, No 3 March 1992

the mouse renin gene promotes transcription in response to 8-bromo-cAMP. Accordingly, in the rat glomerulosa cell cultures, cAMP possibly stimulates renin production through the enhancement of renin gene transcription. We also showed that Ang II inhibited both ACTH- and db-cAMP-stimulated renin production, whereas high potassium-stimulated renin production was not inhibited. These findings suggest that Ang II inhibits a specific action of intracellular cAMP on renin production.

We have reported that bilateral nephrectomy increases adrenal renin, which is caused by the increased levels of plasma potassium and ACTH, and that the infusion of Ang II inhibits the stimulation of adrenal renin in the rat in vivo. Our present results confirm these previous data and suggest that the inhibition of renin production after Ang II infusion in nephrectomized rats is caused by the effect of Ang II on ACTH-stimulated renin production.

The synthesis of cAMP is regulated by adenylate cyclase. Woodcock and Johnston and Hausdorff et al showed that Ang II inhibited adenylate cyclase activity in the glomerulosa cells, and this inhibitory action of Ang II was mediated by the inhibitory guanine nucleotide regulatory protein. These findings suggest the possibility that the inhibitory effect of Ang II on ACTH-stimulated adrenal renin production might be due, in part, to inhibition of cAMP production in the glomerulosa cells. However, our results show that the major inhibitory effect of Ang II is distal to cAMP production because the stimulatory effect of exogenously added db-cAMP is inhibited by Ang II as well as the effect of ACTH.

In conclusion, the effect of Ang II on adrenal renin is different from the kidney. Ang II alone has relatively weak stimulatory effect on renin production in the glomerulosa cells of the rat. On the other hand, Ang II has an inhibitory effect on ACTH-stimulated adrenal renin. The present results suggest that the inhibitory effect of Ang II is in the distal pathway beyond cAMP production. The inhibition of bilateral nephrectomy-stimulated adrenal renin by Ang II infusion in vivo is possibly caused by this inhibitory effect of Ang II on the ACTH action.

References


4. Nakamura M, Misano KS, Naruse M, Workman RJ, Inagami T: A role for the adrenal renin-angiotensin system in the regulation of
Yamaguchi et al  Effect of Angiotensin II on Adrenal Renin  269


Effect of angiotensin II on renin production by rat adrenal glomerulosa cells in culture.
T Yamaguchi, R Franco-Saenz and P J Mulrow

Hypertension. 1992;19:263-269
doi: 10.1161/01.HYP.19.3.263

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/19/3/263

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/