Localization of Components of the Renin-Angiotensin System within the Kidney and Sustained Release of Angiotensins from Isolated and Perfused Kidney

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Inagami, T., Mizuno, K., Kawamura, M., Okamura, T., Clemens, D.L. and Higashimori, K. Localization of Components of the Renin-Angiotensin System within the Kidney and Sustained Release of Angiotensins from Isolated and Perfused Kidney. Tohoku J. Exp. Med., 1992, 166 (1), 17-26 — We found colocalization of renin, angiotensin (Ang) I and Ang II in juxtaglomerular (JG) cells of the kidney. Coexistence of Ang II in renin granules was demonstrated by electron microscopic immunogold labeling of these components. Coexistence of both Ang I and Ang II in the high density renin storage granules were also demonstrated by gradient centrifugation of renal homogenate. These findings supported the synthesis of Ang I and Ang II in juxtaglomerular cells. Isolated and cultured JG cells showed the synthesis of Ang I, Ang II and renin. Ang I and Ang II were secreted from isolated and perfused rat kidneys at steady rates over 2 hr. Their secretion rates were proportional to that of renin. The rate of Ang II secretion from the kidney was higher than that from the vascular bed. Ang II was also found in renal lymph. These findings indicate that a large amount of Ang II is generated in JG cells by the intracellular action of renin and may play a significant role in the regulation of renal function.

Evidence continues to accumulate regarding the roles that intrarenal angiotensin II (Ang II) plays in the regulation of renal functions. To determine the mechanism and site of the intrarenal formation of Ang II, we employed histochemical, cell biological and physiological methods. Immunohistochemical studies have revealed the coexistence of renin and Ang II in juxtaglomerular (JG) cells, and electron microscopic studies and subcellular organelle fractionation have demonstrated the colocalization of renin and angiotensin in renin granules. The mechanism of the accumulation of Ang II in JG granules has been investigated.

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Immunoreactive angiotensin I (Ang I) appeared slowly in JG cells after prolonged administration of angiotensin converting enzyme (ACE) inhibitors. Cloned and cultured renin-containing cells derived from rat kidney were also found to contain renin, ACE, and Ang I and Ang II. The subcellular fractionation of renin granules from rat kidney homogenate demonstrated the presence of Ang I and Ang II in the renin granule fractions. The findings suggest the formation of both angiotensins in JG cells. To study the release of Ang I and Ang II, we determined the release of Ang I and Ang II from isolated rat kidney perfused with Krebs-Ringer buffer at a constant pressure. Release of both peptides was stable for as long as 2 hours in the absence of angiotensinogen in the perfusion medium. There was a positive correlation between renin secretion rate and Ang I secretion rate, and also between Ang I secretion rate and Ang II secretion rate. From these results it has been concluded that Ang II is found in JG cells in the kidney and is directly secreted with renin into plasma or the interstitial fluid, and that Ang II formed in the kidney cells may participate in various renal functions.

Renin is a highly specific protease belonging to the family of aspartyl proteinases. It mediates the first and the rate limiting step in the pathway of the formation of angiotensin II (Ang II) from its prohormone angiotensinogen. The major site of the production and storage of renin is the granular cells of the juxtaglomerular (JG) apparatus. It has been believed that renin is released into the circulation by various specific stimuli and that it begins the production of angiotensins from blood-borne angiotensinogen in plasma. Ang II thus produced plays key roles in the regulation of cardiovascular functions by interacting with cells in vascular beds and the adrenal gland. In addition to the function of Ang II in extrarenal tissues, it has been well known that the various renal functions are also regulated by intrarenal Ang II (Schmid 1962; Thurau 1964; Davis and Freeman 1976; Leyssac 1976; Wright and Briggs 1979; Navar et al. 1982; Navar and Rosivall 1984). Recent studies raised renewed interest in the possibility that locally formed Ang II may participate in the modulation of the renal function. The intrarenal Ang II may be generated in renal circulation. However, evidence has been accumulated for the formation of Ang II in tissue and cells of the kidney. Mendelsohn et al. (1979) reported that the renal tissue Ang II concentrations were much greater than could be accounted for on the basis of circulating Ang II levels. This review is focused on the generation and secretion of Ang II in the kidney.

Immunohistochemical studies

The coexistence of Ang II with renin in rat JG cells was discovered by Celio and Inagami (1981) and Taugner and Hackenthal (1981) with immunohistochemical staining for renin and Ang II in the serial consecutive sections of paraffin-embedded rat kidneys. Immunoreactive Ang II and renin were localized in superimposable JG cells in serial sections (Naruse et al. 1982). A similar
Fig. 1. Serial, consecutive sections of a kidney of a rat chronically fed the ACE inhibitor captopril. A, B, and C contain an identical glomerulus. Positive staining with anti-Ang I antiserum (B) was noted as well as antirenin (A) and anti-Ang II (C) antiserums. Immunohistochemical evidence that angiotensins I and II are formed by intracellular mechanism in juxtaglomerular cells (Naruse et al. 1982) (by permission of the American Heart Association, Inc.).
coexistence of Ang I and renin was found in interlobular arteries of rat kidney (Taugner et al. 1982) and human JG cells (Celio 1982).

These findings suggested two possibilities, namely, the production of Ang II by renin in the same cells by an intracellular mechanism, or the internalization of circulating Ang II from plasma.

To distinguish these alternative mechanisms, we have investigated the presence of Ang I in JG cells. Under normal conditions, Ang I could not be detected in rat JG cells with immunohistochemical staining by using the unlabeled antibody peroxidase-antiperoxidase (PAP) method (Sternberger 1979; Naruse et al. 1981). However, when rats were treated with angiotensin-converting enzyme (ACE) inhibitors (captopril or MK 421) for 2 weeks, Ang I was clearly observed in JG cells in which Ang I and renin were localized, as shown in Figs. 1A, 1B, and 1C. Fig. B shows intense staining for Ang I. However, captopril treatment for 1 week was not sufficient to produce intense Ang I immunoreactivity in JG cells. The prolonged time lag required for the accumulation of Ang I in JG cells was difficult to explain. It is likely that the penetration of ACE inhibitor in JG cells was a slow process. The weak ability of the antibodies against Ang I to stain Ang I in immunohistochemical technique may be another reason. These findings, however, seemed to mitigate against the possible internalization mechanism inasmuch as Ang I in plasma is elevated immediately on administration of ACE inhibitor, and the internalization could occur rapidly after inhibition of ACE.

Immunohistochemical methods were applied to the localization of Ang II and renin at an electron microscopic level (Cantin et al. 1984; Taugner et al. 1984). Antibodies labeled with gold particles were applied to glycol methacrylate- or Lowicryl-embedded rat kidney sections. Ang II immunoreactivity was found in the internum of renin granules along with renin, but no Ang I, angiotensinogen, or ACE was detected by antibodies to these components. Thus, Taugner and his collaborators have postulated that Ang II is not produced in JG cells but may be internalized through a receptor-mediated internalization mechanism (1984).

Renin and angiotensins in renin granules

Renin granules were partially purified from rat kidneys. After differential centrifugation, the P₂ fraction (heavy mitochondrial fraction) was further fractionated on a Percoll density gradient. Renin, Ang I, and Ang II were detected under the same single peak fractions. It is interesting that renin, Ang I, and Ang II all comigrated under the centrifugal force through the Percoll gradient, which indicates coexistence of these three components of the renin-angiotensin system in the same particles (Kawamura et al. 1985). This finding is in agreement with the results of electron microscopic immunohistochemical studies (Cantin et al. 1984; Taugner et al. 1984) in which both renin and Ang II were colocalized in renin granules. In addition, the fractionation studies indicate the presence of Ang I in the same fraction.
Ang II Secretion from Kidney

To further extend our knowledge of the buildup of Ang II in the kidney, cloned and cultured JG cell lines were employed for the study of renin and angiotensins. Kidney cells were dispersed from newborn rat kidneys, and cloned cell lines were established by Rightsel et al. (1982). Cell lines possessing morphologic characteristics of JG cells, such as granular structures and myofibrillar components, were selected. These cells could be distinguished from fibroblasts morphologically.

It is known that the renin-producing ability of JG cells decreases rapidly in culture. The cloned rat JG cells contained renin at a low level; however, it was a measurable level and could be clearly distinguished from the nonspecific renin-like activity of cathepsin D-like enzymes. The pH optima of this renin’s enzyme activity was between 6.5 and 7.0.

It is interesting that these cells were found to contain both Ang I and Ang II, as shown in Table 1. They were also shown to contain ACE at a low level. Thus, these JG cells in culture seem to contain all the apparatus to produce Ang II within the cells like neuroblastoma cells (Okamura et al. 1981).

Renin was found in renal lymph (Proud et al. 1984) at concentrations 20–30 times as high as that of the plasma level of renin. Renal lymph was also found to contain Ang I and Ang II (Proud et al. 1984). It is likely that the renin in renal lymph was released from JG cells in the cortex.

The presence of angiotensinogen in kidney homogenate has been reported previously (Morris and Johnston 1976). Further, angiotensinogen mRNA had been detected recently in the kidney tissue (Campbell and Habener 1987), which indicates that angiotensinogen is also produced locally in the kidney. These observations suggest that Ang II is generated, and released to perform its functions in the kidney.

**Renin and angiotensins release from the kidney**

The above hypothesis was tested in an open perfusion system of isolated

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**Table 1. Renin and Ang I and Ang II/III in cultured cells**

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<tr>
<td>Renin activityd (pg Ang I/hr/10⁶ cells)</td>
<td>25.8 ± 3.7</td>
<td>87.4 ± 16.1**</td>
</tr>
<tr>
<td>% of total renin-like activityd</td>
<td>65.2 ± 2.9</td>
<td>76.3 ± 3.3</td>
</tr>
<tr>
<td>Ang I (pg/10⁶ cells)</td>
<td>84.5 ± 4.6</td>
<td>193.1 ± 26.6</td>
</tr>
<tr>
<td>Ang II/III (pg/10⁶ cells)</td>
<td>36.7 ± 9.1</td>
<td>63.4 ± 2.0*</td>
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</table>

*All values are means ± s.e. (n = 3). *Cells were grown in the presence of 10% fetal calf serum. *Cells were placed in serum-free medium 24 hr before harvest.

*Antibody-inhibitable portion of the total renin-like activity.

*<i>p</i> < 0.05; **<i>p</i> < 0.01.
Fig. 2. Time course of Ang II release from isolated perfused rat kidneys (n = 9). Each column shows the mean ± s.e. value of the release of Ang II for 20 min.

Fig. 3. Time course of Ang I release from isolated perfused rat kidneys (n = 9). Each column shows the mean ± s.e. value of the release of Ang II for 20 min.
Fig. 4. Correlation between baseline renin secretion rate and that of Ang I secretion rate in isolated perfused rat kidneys. Although the variability of baseline releases of renin and Ang I was considerable between different kidneys for unknown reasons, there was a positive correlation between the two variables.

\[ y = 0.406x + 6.940 \]
\[ r = 0.671 \]
\[ p < 0.0001 \]

Fig. 5. Correlation between baseline Ang I secretion rate and that of Ang II secretion rate. There was a highly significant correlation between them.

\[ y = 0.19x + 15.94 \]
\[ r = 0.78 \]
\[ p < 0.0001 \]
kidneys. The isolated rat kidney was perfused with Krebs-Ringer buffer at a
contant perfusion pressure of 110 to 120 mmHg. As shown in Figs. 2 and 3, the
rate of release of angiotensins was as high as 1313.5 ± 184.5 and 772.4 ± 82.5 pg for
Ang I and Ang II, respectively, for the first infusion period of 20 min. The rates
of release of Ang I and Ang II remained constant up to 2 hr. The results seem to
provide experimental evidence for intrarenal production of Ang II as it was
generated continuously in the absence of external supply of angiotensinogen in
the perfusion medium.

To further address the mechanism of intrarenal production of Ang II, we
evaluated the relationship between secreted renin concentration and released Ang
I in the perfusate. As shown in Fig. 4, there was a positive correlation between
the two variables, which suggests that Ang I is generated through intrarenal
pathway of renin-angiotensinogen reaction. Further, there was also a good
positive correlation between the amount or released Ang I and that of Ang II (Fig
5). Although these data are only preliminary, the hypothesis that Ang II is
produced in association with changes in renin secretion may be supported by
recent in vivo studies (Navar and Rosivall 1984; Rosivall et al. 1985) in which
they demonstrated that Ang II formation within the dog kidney could occur in
parallel with enhanced renin secretion due to renal artery constriction.

We had investigated Ang II release from Krebs-perfused vascular beds such
as rat mesenteric artery (Nakamaru and Jackson 1986) and hindquarter leg
(Mizuno et al. 1988). Ang II release from the perfused kidney was much greater
than that from the mesenteric or hindleg vascular beds (Table 2). Renin and Ang
II are also secreted into the renal lymph (Proud et al. 1984).

In summary, although these studies are still in progress, it is clear that JG
cells contain angiotensin, and that Ang II is directly released from the kidney to
play diverse functions in intrarenal regulation.

<table>
<thead>
<tr>
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<th>Ang I (pg/ml)</th>
<th>Ang II (pg/ml)</th>
<th>Flow rate (ml/min)</th>
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<tbody>
<tr>
<td>Kidney (n=32)</td>
<td>23.7±3.8</td>
<td>16.6±3.1</td>
<td>3.4</td>
</tr>
<tr>
<td>Mesenteric vessel (n=18)</td>
<td>N.D.</td>
<td>0.1±0.02</td>
<td>4</td>
</tr>
<tr>
<td>Hindquarter (n=36)</td>
<td>7.9±3.1</td>
<td>4.5±1.3</td>
<td>4</td>
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Value ± s.e. Data was compiled from Refs. 25, 26.
N.D., not detectable.
Ang II Secretion from Kidney

References


