Juxtaglomerular Cells Grown as Monolayer Cell Culture Contain Renin, Angiotensin I-Converting Enzyme, and Angiotensins I and II/III

Wilton A. Rightsel, Tomio Okamura, Tadashi Inagami, James A. Pitcock, Yukio Takii, Bennie Brooks, Peggy Brown, and E.E. Muirhead

From the Departments of Pathology, Baptist Memorial Hospital and University of Tennessee Center for the Health Sciences, Memphis, Tennessee, and the Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee

SUMMARY. A monolayer cell culture of juxtaglomerular cells (JGC) was derived from the renal cortex of neonatal rats. The JGC had the characteristics of those within the kidney, including peripheral dense bodies and myofibrils indicating a smooth muscle origin; rough ER containing fluffy material consistent with protein synthesis; a prominent Golgi apparatus for packaging granules, and granules having the characteristics of secretory granules and lysosomes. Transplants of the cultured cells into syngeneic recipients survived for 2 weeks or longer and retained the features of JGC. The JGC granules fluoresced when treated with a rabbit antibody against pure rat renin, followed by fluorescein isothyocyanate conjugated F(ab')2 fragment of goat antirabbit IgG (Fc fragment) heavy chain specific. The latter indicated the presence of renin. The JGC were lysed in the presence of DFP, captopril, leupeptin, and EDTA, and were extracted in the presence of pepstatin. The lysate contained renin activity that was inhibited by a specific renin antibody. Nonspecific proteases were excluded by the antibody and its pH optimum. Angiotensin I-converting enzyme was detected in the lysate prepared without the use of EDTA and captopril. Angiotensins I and II/III were derived from the extract by additional extractions, TLC, and RIA, using highly specific antibodies. The angiotensins were confirmed by chromatography monitored by authentic angiotensins. We concluded that the cultured JGC contained renin, angiotensin I-converting enzyme, and angiotensins I and II/III. (Circ Res 50: 822-829, 1982)

A MONOLAYER cell culture having the major characteristics of the juxtaglomerular cells (JGC) of the kidney was derived from the renal cortex of neonatal rats, following a suggestion made by Khayat [Khayat et al., 1978 (abstract)]. The JGC were obtained from the syngeneic rat line, Wistar/GM. With this source, it was possible for us to retransplant the cultured cells subcutaneously into normal, mature rats and have a reasonably prolonged survival of the transplant. Reculturing of these transplants, plus a cloning technique, developed a pure cell culture.

Now, we describe the derivation of the JGC culture, the cells themselves, the transplants of these cells, and indicate that these cells contain renin, angiotensin I-converting enzyme, and angiotensins I and II/III.

Methods

Isolation and Establishment of the Cell Culture of JGC

Isolation

Neonatal rats, 24–48 hours old, from the 65th generation of the inbred Wistar/GM rat strain, were anesthetized with ether and their kidneys excised. The tissue-like capsule was removed and the cortices were dissected from each kidney. The pooled cortices were minced finely with iris scissors, and we dissociated the cells by treating the minced tissue with 100 ml of 0.25% 1:250 Difco trypsin in a 250-ml Bellco spinner flask with magnetic stirrer for 2 hours at 37°C. At 30-minute intervals for 2 hours, 50 ml of the trypsinized cell suspension were removed and replaced with an equal volume of fresh 0.25% trypsin. The cell suspension was centrifuged for 3 minutes at 275 × g. The supernatant was discarded, and the packed cells were resuspended in 10 ml of fresh tissue culture medium. The flask was incubated at 37°C for an additional 5 hours. The flask was then centrifuged at 275 × g for 3 minutes. The cells were resuspended in 10 ml of fresh tissue culture medium, and 5 ml were inoculated into each of two 75-cm² tissue culture flasks containing 25 ml of tissue culture medium.

Culture of Dissociated Cortical Cells

Four 5-ml cell suspensions were pooled and centrifuged for 3 minutes at 275 g. The packed cells were resuspended in 10 ml of fresh tissue culture medium. Five milliliters of the suspension, containing approximately 2 × 10⁶ cells, were inoculated into each of two 75-cm² tissue culture flasks containing 25 ml of tissue culture medium. The flask was incubated at 37°C for 2 hours. The cells were then transferred to a second 75-cm² tissue culture flask, and the process was repeated.

Subculture of Dissociated Cortical Cells

Cell suspensions were prepared according to the method of Lewis et al. (1973). We decanted the medium of secondary cultures and removed the cortical cells by treating them
with 20 ml of 0.25% trypsin while incubating them at 37°C in a stationary position for 30 minutes. Then the trypsinized cell suspension was decanted into a centrifuge tube for centrifugation at 275 g for 3 minutes. The supernatant was discarded and the packed cells were resuspended in 10 ml of tissue culture medium. Approximately 5 ml of the cell suspension were inoculated into each of two 75-cm² tissue culture bottles. The cells were subcultured at a ratio of 3:1 to 4:1 for 26 serial passages, after which they were transferred at a ratio of 6:1 to 8:1.

Freezing and Storage Technique

Cells in early transfers were prepared for freezing and storage. A cell suspension containing 6 million cells/ml was prepared in tissue culture medium to which was added 10% calf serum. Aliquots of 1 ml were placed in glass ampules, then sealed and frozen rapidly in liquid nitrogen, using a Linde BF-5 biological freezer. The ampules were stored in a liquid nitrogen refrigerator. To initiate new cultures from the frozen cells, the ampule was rapidly thawed at 37°C and the entire contents were inoculated into a 75-cm² Wheaton flask containing tissue culture medium.

Cloning Method

A cell suspension was used to prepare serial 10-fold dilutions in tissue culture medium. Sufficient dilutions were made so as to obtain a suspension that would contain only one cell/ml. By this limiting dilution technique, 1-ml aliquots of each serial 10-fold dilution were inoculated into replicate tissue culture tubes which were tightly stoppered and incubated at an angle in a stationary position at 37°C. Cultures were examined daily, and only those tubes which contained one cell, as confirmed by microscopic observation, were used for study. When colonies of cells developed in only the single area from a single cell, the culture was transferred as a clone and was assumed to be derived from a single cell. Fourteen clones were isolated and propagated by this limiting dilution method.

Chromosome Analysis

Cells in the 51st transfer were used for chromosome analysis. Thirty-three metaphases of good quality were analyzed. The karyotypes of the diploid metaphases secondary to long-term culture with a clustering around the diploid number having a modal of 42 chromosomes. The karyotypes of the diploid metaphases were basically normal. The metaphases contained XY sex chromosomes.* Thus, the cells in the 51st transfer were of a normal diploid karyotype for the rat.

Recycling (Reculture) of JGC Transplants

Transplants of JGC were removed within the first 2 weeks after their introduction. These were cleaned carefully of adjoining tissue and washed in approximately 5 ml of medium. The nodule was transferred to a sterile petri plate containing 2 ml of complete growth medium. The nodule was minced into minute particles by means of curved scissors. The contents of the petri plate were transferred to a 250-ml tissue culture bottle containing about 30 ml of medium. This was incubated at 37°C and checked daily for growth. When a ⅔ monolayer was formed, the cells were transferred to another container by routine subculture methods. This monolayer was labeled as a reculture of the original isolate.

Monolayer Tissue Culture of JGC and JGC Transplants

A plastic coverslip preparation of the cultured JGC was prepared as previously described (Muirhead et al., 1972) and stained with H & E and toluidine blue. The early phase of the culture formed whorls on the glass. The cells were also examined by EM as previously described for renomedullary interstitial cells (Muirhead et al., 1972).

The transplants of JGC removed at 6, 9, and 12 days were examined microscopically by the same techniques used to examine the cultured cells.

Fluorescent Microscopy

The indirect fluorescent antibody technique was used in an attempt to demonstrate renin-containing granules in the cytoplasm of the cultured JGC. Slides were prepared from tissue culture by growing cells in an eight-chamber tissue culture slide tray with lids obtained from Lab-Tek Products. Each of the eight chambers was inoculated with 5 x 10⁶ cells. These were incubated in an atmosphere of air and 5% CO₂ for 2 days using standard medium. After removal of the chamber coverslips, the slide containing the cells was rinsed with phosphate-buffered saline (PBS). Other slides were prepared from tissue culture transplants by exciting the nodule and then preparing thin frozen sections. The tissue culture slide and the frozen section slide were processed similarly by fixing in 95% methanol at −20°C for 30 minutes, then air-dried. These slides were then stored frozen at −76°C. To each well of the fixed slides or to the tissue section slide was added either a 1:5 or a 1:10 dilution of anti-rat renin antiserum made in the rabbit. (See below for the description of its specificity.)

The slide preparation was held at room temperature for 2 hours. As a control, a 1:5 and a 1:10 dilution of normal rabbit serum was added to a replicate chamber. The slides were washed three times with PBS and then once with distilled water. A 1:64 dilution of fluorescein isothiocyanate (FITC) conjugated F(ab')² fragment goat anti-rabbit IgG (Fc fragment) heavy chain specific was added. The slides with the conjugate were incubated for 30 minutes at room temperature. The slides were washed two times with PBS, counterstained for 5 minutes at ambient temperature with 0.1% Evans Blue, and washed one time with distilled water. Mounting fluid consisting of buffered glycerol was added, then a coverslip placed on the slide which was read with a Leitz Orthoplan research microscope using a dark field oil immersion condenser and a fluorescein oil immersion 54X objective. The light source was a high-pressure 200-watt mercury burner HBO 200. The system contained a K500 barrier filter with BG 38 absorbing filter and a BG 12 excitation filter.

Extraction of JGC

The JGC (10⁶ cells at 12th passage) were cultured in T75 Falcon flasks in basal medium Eagle's containing 0.25% lactalbumin hydrolysate, 0.05% yeast extract, and 10% fetal calf serum, pH being maintained with 4 mM sodium bicarbonate and 25 mM HEPES buffer at 7.2-7.4. As the culture reached confluency in 7-10 days, the medium was changed.

* Seven separate cell lines were eventually derived. Some were of the female (XX) type. Biologically, both lines were identical in the results yielded.

† The goat anti-F(ab')² fragment was pre-titered on JGC to determine the optimal dilution, assuring maximal fluorescence and minimal nonspecific fluorescence.
either to the serum-free basal medium Eagle's or fresh serum-containing medium 24 and 48 hours prior to harvest to determine the effect of serum. Cells were detached from culture flasks with 0.25% trypsin and 1 mM EDTA, washed once with serum-containing medium to inhibit trypsin, then twice with serum-free medium, suspended in water containing a mixture of 1 mM diisopropylfluorophosphate (DFP), 1 mM captopril (Squibb), 5 μg/ml leupeptin (Protein Research Foundation), and 5 mM EDTA, lysed by five cycles of freezing and thawing and then treated with 0.1% Triton X-100. Cell extract used for angiotensin determination was prepared as above in the presence of 1 μM pepstatin (Protein Research Foundation). For the assay of angiotensin I-converting enzyme, cells were released in a similar manner but without using captopril and EDTA. Instead of the detergent Triton X-100, Nonidet P-40 was used for lysing cells and releasing the enzyme.

Renin Activity Assay

Aliquots of cell extract were incubated for 6 hours at 37°C with plasma of bilaterally nephrectomized rats, used as renin substrate, in 0.2 M sodium maleate buffer, pH 6.0, containing 5 mM phenylmethanesulfonylfluoride, 10 mM EDTA, and 0.1% neomycin. The reaction was stopped by heating in boiling water for 10 minutes, and the generated angiotensin I was determined by radioimmunoassay (Haber et al., 1969). As a control for interference by nonspecific renin-like activity of proteases (Day et al., 1976; Hackenthal et al., 1978), parallel experiments were run with the cell extract preincubated with specific antisera to pure rat renin (Motaba et al., 1978) at 1:10^4 dilutions for 16 hours at 4°C. At this dilution, the antisera were found to inhibit completely 10 ng of renin but not cathepsin D activity of rat renal extract (Naruse et al., 1981). The pure rat renin used for producing the antibody was shown to satisfy multiple criteria of purity (Matoba et al., 1978). Furthermore, this antibody was shown to give a single precipitin band by double immunodiffusion. It did not cross-react with human renin and reacted exclusively with JGC when used for the immunohistochemical localization of renin-containing cells in rat kidney sections (Naruse et al., 1981). The pH dependence of renin activity was determined in the following 0.2 M buffers: sodium acetate for pH 3.5-5.0, sodium maleate for pH 5.0-6.5, and sodium phosphate for pH 6.5-7.5.

Angiotensin I-Converting Enzyme Assay

Angiotensin I-converting enzyme activity was determined by a modification of the method of De Pierre and Roth (1975). Sample solutions were incubated with the synthetic substrate hippuryl-His-Leu for 30 minutes at 37°C in 0.4 ml of 50 mM phosphate/50 mM borate buffer, pH 8.0, containing 0.2 M NaCl. The extent of the hydrolysis of

![Figure 1](http://circres.ahajournals.org/)

**Figure 1.** EM of JGC in cell culture. A: A low power (X1600) view of the cells emphasizing the granules in the cytoplasm. B: A higher magnification (X8000) of a single cell pointing out three prominent organelles: rough ER containing fluffy material (arrows 1), the granules (arrows 2), the Golgi apparatus (G). C: The peripheral dense bodies (arrows 1) and myofibrils (arrows 2) are demonstrated (X7,100). D: The acid phosphatase stain (X11,000) is shown in an otherwise unstained section. Few granules are acid phosphatase positive and, therefore, consistent with lysosomes (arrow 1). Most granules are negative. The finely supplied nature of the secretory-type granules and the membrane binding them can be seen at arrow 2 (Barajas, 1979).
FIGURE 2. JGC in the transplant. A: A light microscopy section (X100), stained with H & E, showing the well-packed nature of the JGC in the transplant. At the top, the transplant is pushing against skeletal muscle fibers. The arrows single out capillaries within the transplant. B: A light microscopy of a thick section (0.6 μ) stained with toluidine blue (X750). The granules in the cytoplasm are evident. C: An EM photograph at lower power (X1900) showing two types of granules, a dense granule (arrows 1) and a pale granule (arrows 2). Such mixture of granules has been described for JCC in the kidney (Barajas and Latta, 1963a). At arrow 3, the cytoplasm of adjacent cells interdigitate as also described for JGC in the kidney (Barajas, 1979). D: A higher magnification of EM section (X6,000) emphasizing the peripheral dense bodies (arrows #1) and myofibrils in the inset. Interdigitation is shown at arrow 2.

the hippurylhistidyl peptide bond was determined by fluorometric assay using O-phthalaldehyde. Inhibition of the reaction by 12 nM captopril, 2.3 mM EDTA, or by omission of NaCl from the incubation mixture was examined. The captopril solutions were freshly made prior to use.

Angiotensin Determination

Aliquots of cell extracts were treated with three volumes of acetone, centrifuged at 6,000 rpm for 10 minutes. Supernatant was separated and dried under nitrogen stream at room temperature, redissolved in 0.1 M Tris-acetate buffer, pH 7.4, heated in boiling water, and subjected to radioimmunoassay of angiotensin I (Haber et al., 1969) and angiotensin II (Gocke et al., 1968). The angiotensin I antiserum, supplied by Dr. Robert J. Workman, showed less than 1% cross-reactivity with angiotensin I but 100% cross-reactivity with heptapeptide (angiotensin IIa) and hexapeptide (angiotensin IIa,3), and pentapeptide (angiotensin IIa,3) (purchased from Protein Research Foundation, Osaka, Japan). Greater than 90% recovery of 1 ng of angiotensin I and angiotensin II/III was obtained as tested with these peptides added to the cell extract and treated in the same way. Identity of angiotensins in the extract was confirmed by chromatography on cellulose plates (Analtech) by modification of the method of Semple et al. (1980), using sec-butanol-3% ammonia 5: (vol/vol). After developing the plate, areas containing angiotensins, as determined in reference to standard angiotensins developed in adjacent channels, were scraped, extracted in methanol-ammonia 2:1, vol/vol dried down under nitrogen stream, redissolved in Tris-acetate buffer, pH 7.4, heated in boiling water, and subjected to radioimmunoassay.

Results

Light Microscopy

The tissue cultures that yielded the best JGC in later passages formed whorls in the early passages. In later passages, the cells grew out as a conventional monolayer. The cells had abundant granules in their cytoplasm.

The transplants consisted of similar cells surrounded by capillaries. The appearance was that of an endocrine-type gland.

Electronmicroscopy (EM)

At low power, it can be seen that the JGC of the cell culture contained granules comparable to secretory granules (Fig. 1A). Additional features were evi-
FIGURE 3. (Fluorescent microscopy of JCC transplant). A: The cells display heavy fluorescence toward the FITC-conjugated anti-renin antibody. Some of the fluorescence is granular (upper right and lower left cells); some is diffuse. This is in keeping with granules containing renin and a disruption of such granules with diffuse scatter over the cytoplasm. B: This figure demonstrates absence of fluorescence when the cells were treated with normal rabbit serum (negative control).

dent at higher magnification of isolated cells (Fig. 1B). These included rough endoplasmic reticulum (ER) containing a precipitate of fluffy material suggestive of protein synthesis (Fawcett, 1966), a prominent Golgi apparatus in keeping with the formation of the granules, and peripheral dense bodies and fibrils, consistent with myofibrils, indicative of a smooth muscle origin (Fig. 1C). The acid phosphatase stain (Fig. 1D) demonstrated that most granules were negative for this procedure, while, at the same time, emphasized the finely stippled nature of the inner structure of the specific granules and the membrane binding them. Some granules were acid phosphatase positive, and therefore, consistent with lysosomes. These findings were similar to those described for JGC in situ within the afferent arteriole of the kidney (Lee et al., 1966; Fisher, 1966; Barajas and Latta, 1963a; Barajas and Latta, 1963b; Biava and West, 1966; and Hartroft, 1966 abstract).

The JGC of the transplants displayed the same characteristics of the cells in culture (Fig. 2, A–D). In addition, the overlapping of the cytoplasm of adjacent cells was similar to that described for JGC in the kidney (Fig. 2C).

Fluorescent Microscopy

The granules in the cytoplasm of the cells of the JGC transplants, as shown in Figure 3A, demonstrated an apple-green fluorescence when treated with the FITC-conjugated anti-renin antibody from the rabbit. Controls with normal rabbit serum gave no fluorescence and were negative (Fig. 3B). At least 50% of the cells fluoresced. These results supported the presence of renin in the JGC since the anti-renin antibody was of high specificity.

Renin, Angiotensin I-Converting Enzyme, and Angiotensins I and II/III

Renin activity, angiotensin I-converting enzyme activity, and angiotensins were determined in cell extracts obtained by the combination of hypotonic shock, freezing-thawing cycles, and detergent treatment. As shown in Table 1, demonstrable levels of renin activity sensitive to specific renin antibody and angiotensin I and II/III were present in the JGC grown under normal culture conditions in the presence of 10% fetal calf serum.

Cells harvested from serum-containing medium showed considerable reduction in renin and angiotensin levels upon washing with serum free medium. However, cells harvested 24–48 hours after withdrawal of the serum had negligible amounts of renin and angiotensins that could be readily removed by repeated washing with the serum-free culture medium. Those not removed by repeated washing were considered to be produced in cells. Withdrawal of

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Renin and Angiotensin I and II/III in JGC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Renin activity* (pg Ang I/hr per 10⁶ cells)</td>
</tr>
<tr>
<td>+ Serum†</td>
<td>25.8 ± 3.7</td>
</tr>
<tr>
<td>− Serum‡</td>
<td>87.4 ± 16.1§</td>
</tr>
</tbody>
</table>

All values are means ± se (n = 3).

* Antibody-inhibitable portion of the total renin-like activity.
† Cells were grown in the presence of 10% fetal calf serum.
‡ Cells were placed in serum-free media 24 hrs prior to harvest.
§ P < 0.01.
¶ P < 0.05.
FIGURE 4. The pH profile of angiotensin I-generating activity of the JGC. Angiotensin I-generating activities of cell extracts were determined after preincubation of the extracts with and without (open circles) anti-rat renin antibody at 1:10,000 dilution. The difference of the two values (closed circles) was plotted as antibody-sensitive renin activity which is considered as specific renin activity.

Serum from culture medium for 48 hours resulted in three 4-fold and two 3-fold increases in renin and angiotensin II levels, respectively, in the cells. A large part of the total renin-like activity, as determined by generation of angiotensin I from rat plasma angiotensinogen, was inhibited by the specific antibodies to renin. This antibody-sensitive portion was considered to be renin. A measurable amount of antibody-insensitive renin-like activity was present in the extract. The pH profile of the sum of the antibody-sensitive and insensitive activities (open circles in Fig. 4) showed an optimum in acidic pH. However, the activity inhibitable by the antibodies had an optimum pH between 6.0 and 6.5, in agreement with that of rat renin isolated from the kidney (Matoba et al., 1978). In view of its characteristic pH activity profile and inhibition by the specific antibody, this activity was considered as renin activity, in contrast to nonspecific renin-like activity presumably due to protease (Day et al., 1976; Hackenthal et al., 1978).

This renin activity was found to decay slowly but steadily over a period of 3-6 weeks, during which 3-6 additional passages were made. After 6-12 additional passages, specific renin activity inhibitable by specific antibody was no longer detectable. On the other hand, the nonspecific renin-like activity which was insensitive to the antibody and had an acidic pH-activity optimum, increased steadily.

Renin activity in serum-free culture medium was determined by the same method at the end of the 15th passage. An activity of 32.6 ± 6.2 pg angiotensin I/h per 10^6 cells was approximately 30% of the activity remaining in the cells.

Of renin activity in the medium, 98.7 ± 1.3% was inhibitable by the antibody and this activity showed a neutral pH optimum, indicating that the renin-like activity released from cells was almost exclusively specific renin.

Angiotensin I converting enzyme-like activities measured by using synthetic substrate are shown in Table 2. This hippuryl-His-Leu hydrolyzing activity was almost completely inhibited by the addition of 2.3 mM EDTA or 12 nM captopril, a specific inhibitor of angiotensin I-converting enzyme. A strong dependence of the enzyme activity on chloride ion concentration was also observed as an evidence for the identity of angiotensin I-converting enzyme. Specific activity of angiotensin I-converting enzyme was affected little by the withdrawal of fetal calf serum in the medium.

Immunoassayable angiotensins characterized by thin layer chromatography indicated that substance detected by angiotensin I radioimmunoassay had chromatographic mobility identical with synthetic angiotensin I, whereas substance detected by angiotensin II assay consisted of angiotensin II and III as examined by thin layer chromatography.

Discussion

The present study entailed the derivation of cells having the characteristics of JGC from newborn rat kidney. The morphological features included secretory-type granules, lysosomes, a Golgi apparatus, rough ER-containing precipitated, fluffy material and myofibrillar structures attached to peripheral dense bodies. The demonstration that these cells contained both renin, angiotensin I-converting enzyme, and angiotensins I and II/III supported further the JG nature of the cells. Furthermore, the fact that as much as 30% TABLE 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>Angiotensin I-converting enzyme-like activity (pmol His-Leu/min per 10^6 cells)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells (serum)*</td>
<td>76.1 ± 12.1</td>
<td>97</td>
</tr>
<tr>
<td>+ 2.3 mM EDTA</td>
<td>2.2 ± 0.2</td>
<td>92</td>
</tr>
<tr>
<td>+ 12 nM captopril</td>
<td>5.9 ± 0.5</td>
<td>92</td>
</tr>
<tr>
<td>+ Omission of NaCl†</td>
<td>1.0 ± 0.09</td>
<td>99</td>
</tr>
<tr>
<td>Cells (serum-free)‡</td>
<td>76.5 ± 8.5</td>
<td></td>
</tr>
</tbody>
</table>

* Cells were grown in the presence of 10% fetal calf serum.
† The 0.2 mM NaCl was omitted.
‡ Cells were placed in serum-free media 24 hours prior to harvest.

All values are means ± SE (n = 3).
of the total renin was in the culture medium was in contrast to extrarenal renin-containing cells such as neuroblastoma cells in culture. These cells retain practically all renin within the cells (Okamura et al., 1981). These findings also seemed to reflect the secretory capability of JGC. The encountering of renin and angiotensin I-converting enzyme, together with angiotensins I and II/III, in thoroughly washed cells indicated that the angiotensins were formed by an intracellular mechanism.

Repeated washing removed only part of renin and angiotensins from cells grown in serum-containing medium. Moreover, cellular renin and angiotensin levels increased markedly after withdrawal of serum. Both of these findings suggest strongly that unwashable renin and angiotensins which become measurable only after cell lysis were endogenous to cultured cells but were not due to endocytosis by cells that had fetal calf serum in the medium. On the other hand, the origin of angiotensinogen is not clear. Although it is possible that JG cells internalized and utilized angiotensinogen in the serum used in the culture medium, the increase in angiotensins induced by withdrawal of the serum also suggests the possibility of endogenous synthesis of the prohormone.

The presence of tightly bound angiotensin I-converting enzyme, and lack of effect of the withdrawal of serum for 48 hours, also suggests the endogenous nature of this enzyme. The complete loss of the enzyme activity to hydrolyze the synthetic substrate in the absence of chloride ion and complete inhibition by captopril at a low concentration indicates that this is the angiotensin I-converting enzyme.

The amount of renin and angiotensins in the cells in primary culture was low. The low concentration of renin and angiotensins seemed to be due to a steady decline of the intracellular concentration of these components in primary culture, presumably due to loss of differentiated properties during the well known dedifferentiation of normal cells in primary culture.

The presence of nonspecific renin-like activity could have been misleading. However, lack of inhibition by specific antibodies to renin and the acidic pH optimum were utilized to distinguish nonspecific from specific renin activity. The fact that only the specific enzyme was found in the culture medium indicated that the nonspecific enzyme was released as a result of the exhaustive measures used in disrupting the cells.

The intrarenal generation of angiotensin II has been suggested in the past. However, the demonstration of angiotensins by quantitative assay in the renal extract or even in renal cell suspension medium has not been possible, presumably due to rapid destruction of the peptides. The use of cultured cells and protease inhibitors eliminated the source of angiotensin destroying enzymes and enabled us to detect angiotensins by quantitative assay.

The presence of angiotensin II in renin-containing juxtaglomerular cells has been demonstrated recently by an immunohistochemical study (Celio and Inagami, 1981). The latter observation and the present result complement each other and support a new concept that renin-containing cells have the capability for intracellular synthesis of angiotensins. The intracellular mechanism may be more in line with the general mechanism of peptide hormone formation in other endocrine tissues. Angiotensin formation in neuroblastoma cells seemed to follow this general mechanism (Okamura et al., 1981). The release of renin to plasma from juxtaglomerular cells may be an additional mechanism to the basic intracellular mechanism to meet acute demands for rapid distribution of angiotensin II.

The cultured JGC described in the present report are capable of inducing a hypertensive state when retransplanted into syngeneic recipients and followed by uninephrectomy [Muirhead et al., 1979 (abstract)]. The detailed description of this hypertensive state is being presented separately. Five major features of this hypertensive state are emphasized. The plasma renin level (concentration and activity) remains at baseline level, i.e., nonelevated, during the developmental phase of the hypertension, while the plasma angiotensin II/III is markedly elevated. At the time the plasma angiotensin level is elevated, an infusion of Seralasin lowers the AP to normal levels. Initiating a captopril intake at the time of the JGC transplant prevents the hypertensive state. The hypertension has features of "malignant" hypertension, mainly fibrinoid necrosis and musculomucoid hyperplasia of small arteries and arterioles of the viscera. Thus, the hypertensive state appears due to hyperangiotensionemia in the absence of hyperreninemia. These various features support the concept that the JGC produce angiotensin intracellularly and secrete it either directly into the blood stream (a more likely mechanism) or indirectly via lymphatics (a less likely mechanism).

The presence of angiotensin II in the juxtaglomerular cells suggests a local effect of angiotensin II within the kidney. Locally, secreted angiotensin II may affect renin release by a direct effect on the juxtaglomerular cells (Naftilan and Oparil, 1978), glomerular filtration rate by changing glomerular permeability coefficient (Blantz et al., 1976), or resistance of the efferent arterioles (Davalos et al., 1978), tubular sodium reabsorption, and tubuloglomerular interaction (Leysac, 1976). Renin and angiotensin may be released not only into blood but also to interstitium and lymphatic channels. The renomedullary interstitial cells have receptors for angiotensin II (Brown et al., 1980), and there is a suggestion that angiotensin II may constrain the antihypertensive action of these cells (Muirhead et al., 1981). Thus, local renal angiotensin II could affect the kidney in multiple ways.
We would like to express appreciation for the gift of antibodies to angiotensin II from Dr. Robert J. Workman, and technical assistance by Trinita Fitzgerald, William M. Brosius, and Mary F. Hall.

Supported by U.S. Public Health Service Grants 19287 and HL-14192 from the National Institutes of Health.

Address for reprints: E.E. Muirhead, M.D., 899 Madison Avenue, Memphis, Tennessee 38146.

Received September 14, 1982; accepted for publication February 25, 1983.

References


Day RP, Reid IA (1976) Renin activity in dog brain: Enzymological similarity to cathepsin D. Endocrinology 99: 93-100

De Pfeffer D, Poth M (1979) Fluorometric determination of dipeptidyl carboxypeptidase (angiotensin I converting enzyme). Enzyme 19: 65-70


Juxtaglomerular cells grown as monolayer cell culture contain renin, angiotensin I-converting enzyme, and angiotensin I and II/III.

W A Rightsel, T Okamura, T Inagami, J A Pitcock, Y Takii, B Brooks, P Brown and E E Muirhead

_Circ Res._ 1982;50:822-829
doi: 10.1161/01.RES.50.6.822

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1982 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/50/6/822