# MINIREVIEW

# Origin of the Angiotensin II Secreted by Cells (43699A)

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*Abstract.* **Circulating angiotensin II is unique in that it is formed in the blood by the interaction of circulating proteins. There are in addition many local renin-angiotensin systems in tissues in which angiotensin II is apparently secreted by various types of cells. This brief review considers the possible pathways for synthesis of locally produced angiotensin II in the brain, the anterior pituitary, the testes, the ovaries, the adrenal cortex, the kidneys, the heart, blood vessel walls, and brown and white fat. Synthesis by cells in culture is also reviewed. The possibility that certain cells contain a complete intracellular renin-angiotensin system is not ruled out, but there are problems with this hypothesis. Proteases other than renin may be involved, and there may be different pathways in different tissues. However, it appears that at least in some tissues, angiotensinogen is produced in one population of cells and transported in a paracrine fashion to other renin-containing cells, where it serves as the substrate for production of angiotensin II.** [P.S.E.B.M. 1994, Vol 205]

Circulating angiotensin II (Ang II) is different from other hormones in that it is formed in the blood by the action of circulating proteins: renin, an acid protease, acts on angiotensinogen to form the decapeptide angiotensin I (Ang I) (Fig. 1), and the physiologically active octapeptide AII is then formed by the action of angiotensin-converting enzyme (ACE).

We now know that in addition to the circulating renin-angiotensin system there are many tissue renin-angiotensin systems that appear to produce Ang II for local use independent of the circulating system (1, 2). Cells in these systems contain, and presumably secrete, Ang II; in a few instances, secretion from single cells has been demonstrated (Ganten, personal communication). It has been assumed by a number of investigators that Ang II-secreting cells in these tissues contain a complete intracellular renin-angiotensin system that is responsible for the synthesis of the Ang II they secrete (Fig. 1). However, there are problems with this assumption. One is that in many of the tissues, the various components of the renin-angiotensin system are found in different cells, and it has not been possible to identify all the components of the system in single cells. In addition, cells contain two different secretory pathways, the nonconstitutive or regulated pathway in which proteins are processed in secretory granules before secretion, and the constitutive pathway in which proteins are packaged in vesicles and rapidly released into the interstitial fluid (3). Prorenin must be converted to active renin before it can hydrolyze angiotensinogen, and this activation occurs in the secretory granules (4). There has been speculation that prorenin has some intrinsic activity under special circumstances (5), but in general, this is probably not true, since even though circulating prorenin levels can be normal or elevated after bilateral nephrectomy, the level of active renin in the circulation is zero (6). On

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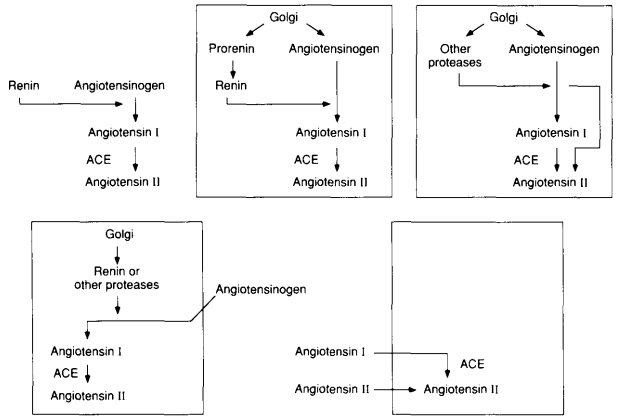


**Figure 1.** Possible pathways for the formation of angiotensin II in cells. ACE, angiotensin-converting enzyme. The boxes represent single cells. Top left: formation of angiotensin II in the circulation. Top middle: complete intracellular renin-angiotensin system. Top right: synthesis by action of other proteases, either via angiotensin I or direct to angiotensin II, bypassing ACE. Bottom left: synthesis from angiotensinogen taken up from extracellular fluid. Bottom right: uptake of angiotensin I or angiotensin II from extracellular fluid.

the other hand, angiotensinogen always appears to be secreted by the constitutive pathway. Further evidence for this separation has been obtained in transfection experiments. When the renin gene is transfected to AtT-20 cells, which are ACTH secreting cells of mouse tumor origin, prorenin is formed and directed to the secretory pathway, where it is activated (7). When the angiotensinogen gene is transfected to these cells, all the angiotensinogen is directed to the constitutive pathway. Since the regulated and the constitutive pathways are separate, it is difficult to see how renin and angiotensinogen could get together inside cells.

Ang II can be formed by acid proteases other than renin, and some of these proteases can form Ang II directly from angiotensinogen, bypassing the ACE step (8, 9). The problem of getting an active protease from the secretory granule pathway to the angiotensinogen remains, but possible synthesis by an enzyme other than renin merits consideration.

A third possibility is that angiotensinogen from other cells is taken up by the Ang II-secreting cells and interacts with renin or other acid proteases. There is precedent for this pathway because a variety of substances, including proteins, have been shown to be taken up by endocytosis, sequestrated in endosomes, and then combined with active proteases in the acid lysosome pathway (9, 10). It is significant in this regard that in rats, the granules of the juxtaglomerular cells contain Ang II as well as active renin and are definitely lysosomal in character (4). Of course, a fourth possibility is that Ang I is taken up and converted intracellularly to Ang II, and a fifth is that Ang II itself is taken up from the extracellular fluid, sequestrated in secretory granules, and released from the cell that took it up. These possibilities are summarized in Figure 1. There are abundant Ang II receptors, primarily of the $AT_1$ type, on juxtaglomerular and other cells (11) that could mediate receptor-mediated endocytosis, and Ang II uptake has been documented in vascular smooth muscle (12). Ang I and angiotensinogen receptors have not been demonstrated, but this does not mean they do not exist.

In this short review, possible mechanisms for the production of Ang II in cells are considered in terms of what is known about Ang II formation in those tissues for which enough information is available to analyze the process. Consideration is also given to cells in culture. The review focuses on Ang II production and secretion, not on the subsequent fate or action of the secreted peptide. Many references are to reviews, which should be consulted for specific references to primary reports.

## Tissues

**The Brain.** The location of the components of the renin-angiotensin system in the brain has been discussed in a number of recent reviews (13–16) and needs only be summarized here. Ang II is present in nerve endings as granular aggregates and in the walls of vesicles, with increased concentrations at the active zones of synapses. Occasionally, it is also found in large granulated vesicles (17; C. F. Deschepper, unpublished observations). The nerve endings are primarily in the hypothalamus and brainstem (18). The peptide has a variety of effects on neurons (19), and it seems very likely that it functions as a neurotransmitter. Renin is present in synaptosomes (20), and renin mRNA is present in the brain (21, 22) although its definite location by *in situ* hybridization has not been reported. Most of the ACE in the brain is located in the brush border of the choroid plexus cells and the circumventricular organs, with lesser amounts in the membranes of some neurons (15). However, the angiotensinogen is in astrocytes, and furthermore, it is primarily located in astrocytes in the brainstem, not in the cerebral cortex. There have been reports that neurons also contain angiotensinogen-like immunoreactivity (23), but angiotensinogen mRNA has only been found in astrocytes when *in situ* hybridization is paired with markers that are specific for astrocytes and markers that are specific for neurons (24). Of course, it is almost impossible to prove a negative, i.e., that there is no angiotensinogen in neurons, but certainly most of the evidence points in that direction.

Thus, the morphological evidence seems most consistent with the hypothesis that angiotensinogen produced by astrocytes is taken up by neurons and converted to Ang II, although extracellular formation of Ang I or Ang II with endocytosis of the peptides remains a possibility.

**Anterior Pituitary.** The distribution of the components of the renin-angiotensin system in the anterior pituitary has also been reviewed on several occasions (7, 15, 25). In rats, Ang II and renin are both located in gonadotropes, and specifically in the secretory granules that contain LHβ (25, 26). These cells also contain renin mRNA and continue to contain Ang II immunoreactivity when the pituitary is organ cultured for 14 days in serum-free medium (7, 26). Most of the ACE of the anterior pituitary is associated with the endothelium of the sinusoids, but some is also found in gonadotropes. The amount of angiotensinogen mRNA in the anterior pituitary is relatively low, but it has been detected in extracts of the gland (27). Angiotensinogen-like immunoreactivity is present in a separate population of cells that does not secrete any of the known anterior pituitary hormones (15, 28). These results suggest that the situation in the pituitary of the rat is similar to that in the brain, i.e., that angiotensinogen secreted by one type of cell is transported in a paracrine fashion to another type, where it is taken up, or possibly serves as the extracellular substrate for Ang I and Ang II which then enter the cells. It is worth noting, however, that in the rat Ang II receptors, which could be responsible for receptor-mediated endocytosis, are located on lactotropes and corticotropes not on gonadotropes (15).

A dissenting view has been expressed by Sernia *et al.* (29). They carried out the important experiment of dispersing anterior pituitary cells on agar and analyzing their output of angiotensinogen by the reverse hemolytic plaque assay. Confirming our results, they found that most of the angiotensinogen-containing cells did not contain LH or prolactin. However, they also found that angiotensinogen was released from some large cells that contained LH. Since gonadotropes did not appear to take up iodinated angiotensinogen, they concluded that angiotensinogen was manufactured in the gonadotropes. The experiment with labeled angiotensinogen needs to be confirmed. The case for synthesis by gonadotropes would be strengthened if angiotensinogen mRNA could be demonstrated in the cells by *in situ* hybridization, but this is difficult because the concentration of angiotensinogen mRNA in anterior pituitary cells is low. In the meantime, it is clear that most if not all the angiotensinogen in the anterior pituitary comes from other cells.

**Testes.** The Leydig cells of the testes contain immunoreactive renin and renin mRNA (30, 31). They also contain immunoreactive Ang I, Ang II, and Ang III (30; C. F. Deschepper, unpublished observations). There are as yet no reports of localization within granules in these cells. The testes are unique in that they contain two forms of ACE (32, 33). One of these, a truncated form with only one active site, has been called germinal ACE. It is located in the seminiferous tubules, and specifically in the heads of sperms (33). The testes also contain somatic ACE, a larger form of ACE with two active sites that is found throughout the body in endothelial cells (33). The somatic ACE in the testes is primarily in the endothelium of the blood vessels, but some is also found in the interstitium, presumably associated with the Leydig cells. On the other hand, angiotensinogen mRNA has not been detected in rat testes (34). In addition, the concentration of angiotensinogen is no greater in testicular venous blood in humans than in arterial blood, although the prorenin concentration is greater (35). Therefore, one could argue that the situation is analogous to that in the brain and the anterior pituitary: the Leydig cells make Ang II with the renin and ACE they contain using angiotensinogen from another source—in this case, from the circulating blood.

**Ovaries.** Human ovarian follicular fluid contains 11.5 times as much prorenin as plasma, 2.5 times as much active renin, and more Ang II. Angiotensinogen is also present, although the concentration is only 60% of the concentration in plasma (36). In biopsies of human follicles and a corpus luteum of pregnancy, Ang II immunoreactivity and renin immunoreactivity have been found in luteinized granulosa cells of preovulatory follicles, thecal cells, stroma, and luteal cells (37). In rats, ACE has been found in granulosa and luteal cells (38), and angiotensinogen immunoreactivity has been found in granulosa and luteal cells (39). We found renin mRNA in the corpus luteum of young rats treated with gonadotropins (40). However, no signal was detected in thecal or follicular cells. In addition, although angiotensinogen mRNA is present in ovarian extracts (41), it has not been localized to a given part of the ovary. Thus, although it seems clear that Ang II is manufactured in the ovary, it is not possible as yet to determine whether one or more than one type of cell is involved in its synthesis.

**Adrenal Cortex.** The adrenocortical renin-angiotensin system has been extensively studied to elucidate its relation to the circulating renin-angiotensin system in the regulation of aldosterone secretion (42). Renin has been found in the zona glomerulosa of the adrenal cortex of rats and humans. Renin mRNA is present in the zona glomerulosa of rats (31), and the renin is located in dense granules (43). Ang I, Ang II, and Ang III are also present in cells of the zona glomerulosa (42). ACE is present by [$^3$H] captopril autoradiography, although the exact cell type has not been determined. Furthermore, preparations of zona glomerulosa cells incubated with Ang I produce Ang II, and this is blocked by ACE inhibitors. However, the mRNA for angiotensinogen is concentrated in fibroblast-like cells in the adrenal capsule, not in steroid secreting cells (44).

Thus, the pattern in the adrenal cortex appears to resemble that in the brain and anterior pituitary: the zona glomerulosa cells appear to synthesize and secrete Ang II. They also contain renin, and ACE is at least nearby. However, angiotensinogen is produced by a different cell type in the adrenal capsule and presumably moves in a paracrine fashion to the cells in the zona glomerulosa that make Ang II.

**Kidneys.** As noted above, the granules of the juxtaglomerular cells that secrete renin into the bloodstream resemble lysosomes, and it is well established that in rats, they contain Ang II in addition to active renin (45–47). Normally, Ang I is not detected in juxtaglomerular granules in rats, but it appears after treatment for prolonged periods with ACE inhibitors (48). It has been argued that this is additional evidence for synthesis of Ang II in the granules as opposed to uptake. However, it could also be explained by uptake of Ang I; the circulating level of Ang I is markedly elevated by inhibition of ACE, and when the enzyme was inhibited, the amount of Ang II in the juxtaglomerular granules was not reduced (48). In addition, ACE is not present in juxtaglomerular granules but is localized in the endothelium overlying the cells. Angiotensinogen also cannot be detected in the juxtaglomerular cells and is found instead, along with its mRNA, in the proximal tubular epithelium (49). These observations, plus the abundant $AT_1$ receptors on the juxtaglomerular cells (11) suggest that Ang II in the juxtaglomerular granules is taken up from the blood by receptor-mediated endocytosis. Uptake of Ang I or angiotensinogen with formation of Ang II in the granules is also a possibility. At present, one can only conclude that it is not possible to distinguish between synthesis and the various forms of uptake. It should be noted in addition that the presence of Ang II in juxtaglomerular granules is largely unique to the rat. Small amounts of Ang II immunoreactivity are seen in the mouse, but none has been detected in the juxtaglomerular granules of humans, pigs, dogs, cats, and four other species in which it was deliberately sought (50).

**Heart.** All the components of the circulating renin-angiotensin system are said to be present in the heart (51). The heart also contains chymase, an enzyme that converts Ang I to Ang II in human hearts and to inactive peptides in rat hearts (52, 53). This enzyme is not inhibited by ACE inhibitors. Angiotensinogen mRNA is present in the heart, but there is a debate about renin mRNA. All agree that the renin mRNA content is low (54), but some find it with the polymerase chain reaction (PCR) (55), whereas others do not. Recently, it has been reported that cultured cardiac myocytes and fibroblasts from neonatal rat hearts contain Ang I, Ang II, ACE, renin, angiotensinogen, and the mRNAs for renin and angiotensinogen (53, 55). These observations, which suggest the presence of a complete renin-angiotensin system in both cell types, are discussed and analyzed below in the section on cultured cells.

**Vascular Tissue.** The components of the circulating renin-angiotensin system are also found in the walls of blood vessels. Initially, it was argued that angiotensinogen mRNA was present in perivascular fat rather than vessel walls (56); however, subsequent reports indicated that this mRNA was present in both locations and that the mRNA in vessel walls, unlike that in perivascular fat, was increased by exposure to a low sodium diet (57). Both vascular smooth muscle cells and endothelial cells have been reported to produce Ang II, as discussed in more detail below in Cultured Cells.

**Brown Fat.** Both brown and white fat are relatively homogeneous tissues, and both have been reported to contain components of the renin-angiotensin system. More details are available on brown than white fat. Angiotensinogen mRNA is present in brown fat, along with active renin, ACE, and Ang I (56, 58). These components are affected by feeding (59). However, it has not been possible to detect renin mRNA, even with the PCR (8). Studies on 3T3-F442A in cultures suggest that these cells, which differentiate into adipocytes, produce Ang II by the action on angiotensinogen of an enzyme that differs from renin.

## Cultured Cells

Cells in culture have also been used to address the question of whether single cells can synthesize Ang II intracellularly and secrete it into the extracellular fluid. It has been claimed that there are "complete renin-angiotensin systems" in neuroblastoma cells, testicular Leydig cells, adrenal cells, juxtaglomerular cells, cardiac cells, vascular smooth muscle cells, and endothelial cells. However, a number of important variables must be considered in evaluating these reports. Cells must be grown in serum-free medium in order to avoid any uptake of components of renin-angiotensin system from fetal calf serum or other forms of blood derivatives added to the culture medium. Primary cultures are often mixed cultures containing more than one cell type. In addition, release by cells of Ang II into the medium does not by itself prove synthesis *de novo* from intracellular renin and angiotensinogen, because, as noted above, other enzymes can catalyze the formation of Ang II.

Established neuroblastoma cells were among the first to be studied. Some of these were shown to contain renin, ACE, and Ang II (60). However, their angiotensinogen content was low or undetectable. It was argued that since angiotensinogen was secreted constitutively its content would be expected to be low. However, angiotensinogen can be readily detected immunocytochemically in astrocytes (see above), ante-

rior pituitary cells (see above), and the liver (61). In a separate series of experiments, Clemens *et al.* (62), found that two of the neuroblastoma cell lines studied by Okamura *et al.* (60) released angiotensinogen into the medium in culture, and argued that this was evidence that they contained angiotensinogen. However, astrocytes are now known to produce angiotensinogen, and astrocytes and neurons come from the same stem cells. Thus neuroblastoma cells might have properties of astrocytes that are absent in adult neurons. In addition, the neuroblastoma cells were grown in angiotensinogen-containing fetal calf serum before transfer to serum-free medium. The case for a complete intracellular renin-angiotensin system would be more convincing if both renin mRNA and angiotensinogen mRNA could be detected by *in situ* hybridization in the same neuroblastoma cell. This colocalization has not been reported.

Rat Leydig cells purified on density gradients contain renin, ACE, and Ang I, Ang II, and Ang III (30). However, angiotensinogen was not detected, and, as noted above, no angiotensinogen can be detected in testicular tissue.

It is difficult to evaluate the reports on adrenal cells because as noted above, angiotensinogen appears to be located in cells of the adrenal capsule rather than the zona glomerulosa cells that contain renin and Ang II. Zona glomerulosa cell cultures that were more than 90% pure produced Ang II (42), but it was not proved these adrenal cell preparations were free of other capsular cells.

There have been various attempts to isolate and grow juxtaglomerular cells in culture. Rightsel *et al.* (63) prepared primary cultures from neonatal rats and then prepared further cultures from single cultured cells. They identified renin, ACE, Ang I, Ang II, and Ang III in their putatively pure cell cultures. However, the cultures were grown in serum-containing medium and were not shown to produce angiotensinogen on their own. In this regard, they resemble gonadotropes of the anterior pituitary and some other cells *in vitro* in having all the components except angiotensinogen. Others have attempted to prepare cell lines from juxtaglomerular cell tumors, but have had trouble with cells dedifferentiating in culture.

The cultured cardiac myocytes and fibroblasts reported by Dostal *et al.* (53, 55) are in some ways the most intriguing. These cells were studied after incubation in serum-free medium for five days. Both cell types were reported to contain renin and angiotensinogen mRNA. In addition, both the cardiac myocytes and the fibroblasts were reported to contain immunoreactivity for renin, angiotensinogen, ACE, Ang I, and Ang II. Confirmation of these observations is awaited with interest. Immunocytochemically identified products in these cells were somewhat unique in being perinuclear in location, rather than peripheral, and in being apparently associated with the cytoskeleton. This is not the distribution of the components of the renin-angiotensin system identified in other cell types *in vivo*. Furthermore, as noted above, some investigators have had difficulty identifying renin mRNA in cardiac tissue, even with the use of PCR.

Eggena *et al.* (64) have reported that cultured rat aortic smooth muscle cells produce angiotensinogen, and Naftilan *et al.* (57) have reported that angiotensinogen mRNA is present in vascular smooth muscle cells. Dzau *et al.* (9) have reported that cultured vascular smooth muscle cells also contain Ang II. On the other hand, Gohlke *et al.* (65) have reported that Ang I is not converted to Ang II in vascular tissue if the endothelium is destroyed, indicating that the vascular smooth muscle cells do not contain ACE activity.

It is well established that endothelial cells contain ACE. Cultured bovine endothelial cells contain renin and Ang I (66). They were also reported to contain a small amount of angiotensinogen (67). The proteins were not found in the culture medium, but the medium contained Ang II and Ang III when the cells were switched to serum-free medium (67). The possibility that the angiotensinogen was taken up from the calf serum in the medium was not ruled out, and the case for a complete intracellular renin-angiotensin system would be strengthened if angiotensinogen mRNA and renin mRNA could be demonstrated in the same endothelial cell.

## Conclusions

The data summarized in the preceding paragraphs make it clear much is still to be learned about how the Ang II secreted by cells in many different tissues is synthesized. Renin and angiotensinogen may be synthesized in the same cell and interact intracellularly to form Ang II, but there are problems with this hypothesis. Alternatively, other intracellular proteases may form Ang II with or without Ang I as an intermediate. A third possibility is that angiotensinogen is taken up from the extracellular fluid and interacts with intracellular proteases in lysosomes or similar organelles, and a fourth is direct uptake of Ang I and/or Ang II. Of course, there may be different pathways in different cells. However, in the brain, the anterior pituitary, the adrenal cortex, the testes, and possibly other organs, localization and other studies suggest that uptake of extracellular angiotensinogen is a real possibility. A careful search for an uptake pathway and for an angiotensinogen receptor would seem to be in order.

This review is dedicated to F. Merlin Bumpus, one of the true pioneers in this field, who died August 8, 1993. The review includes

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