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# Adrenal Renin: A Possible Local Regulator of Aldosterone Production

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Extrarenal renin has been identified in a number of tissues, including the brain, the submaxillary gland, uterus, ovary, vascular endothelium, testes, pituitary gland, and the adrenal cortex. In some tissues, including the adrenal cortex, all of the components of the renin-angiotensin system have been identified; however, no specific physiologic role has been clearly demonstrated for these extrarenal renin-angiotensin systems. We have studied the role of the renin-angiotensin system in the adrenal cortex of the rat and have found that renin is localized and synthesized in the zona glomerulosa cells. Its production can be influenced by alterations in electrolyte balance, as well as the genetic background of the rat. In adrenal capsular explant cultures, a converting enzyme inhibitor can lower angiotensin II production and reduce the stimulation of aldosterone by potassium, suggesting that this system is involved in the aldosterone response to potassium. In addition to rat adrenals, renin has been identified in human adrenal tissue and human adrenal tumors, including aldosteronomas, and a patient with hypertension has been reported to have an adrenal tumor that appeared to be secreting renin into the circulation.

## INTRODUCTION

The renin-angiotensin system is the major physiological regulator of aldosterone secretion and plays an important role in the regulation of blood pressure. Renin is an enzyme secreted by the kidney which acts upon the renin substrate angiotensinogen in blood to form the decapeptide angiotensin I (AI). The latter is converted by angiotensin converting enzyme (ACE) present in most endothelial cells to form angiotensin II (AII). More than 80 percent of the angiotensin I is converted to angiotensin II during one circulation through the lung. AII is the active principle in the system and stimulates aldosterone secretion as well as being a potent vasoconstrictor. Aldosterone enhances sodium reabsorption and potassium excretion by the kidney. Several studies have suggested that unidentified factors may also regulate aldosterone production. A local adrenal renin-angiotensin system may be one such factor.

## EVIDENCE FOR THE PRESENCE OF RENIN IN THE ADRENAL CORTEX

The renin enzyme has been identified in a variety of tissues outside the kidney, specifically, the submaxillary glands, the chorion and uterus, vascular endothelial and smooth muscle cells, the testes, the ovaries, the anterior and intermediate lobe of the pituitary, the brain, and the adrenal cortex [1]. Renin has also been detected in various tumor cell lines such as Leydig cell tumor [2], neuroblastoma cells [3], and prolactino-



*Abbreviations:* ACE: angiotensin converting enzyme AI: angiotensin I AII: angiotensin II RNA: ribonucleic acid SHR: spontaneously hypertensive rat WKY: Wistar-Kyoto rat

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mas [4], as well as adrenal tumors [5]. These data suggest that renin may generate angiotensin in the various tissues and act as a local endocrine system. Although earlier measurements probably include nonspecific proteases, recent investigations have identified specific renin isoenzymes by a variety of techniques, and, in some tissues, all of the components of the renin-angiotensin system are present.

Although the presence of a renin-like enzyme in the adrenal gland was described earlier, in 1967 Ryan [6] presented the first sound evidence for the presence of a renin-like substance in the adrenal gland. His group extracted rabbit adrenal glands, incubated the renin extract with natural substrate at pH 6, and identified the product as angiotensin I. In 1974, Ganten et al. [7] performed a series of experiments demonstrating renin isoenzymes in rat and human adrenal tissue and found that feeding rats a low-sodium diet increased the adrenal renin concentration. He also reported that human aldosteronomas had a much higher adrenal renin concentration than adjacent human adrenal tissue. The methods for measuring renin in those earlier reports did not eliminate the possibility that other enzymes such as cathepsin D may have contributed to the generation of angiotensin I.

In 1981, Aguilera et al. [8], while studying adrenal angiotensin II receptors after nephrectomy, described the presence of a renin enzyme in the outer zone of the adrenal cortex of the rat. They also reported the presence of angiotensin II in the adrenals as well as in the plasma of nephrectomized rats. These investigators suggested that the adrenal cortex may be a source of the plasma AII in nephrectomized rats. In 1982, Mendelssohn [9] reported the extraction of angiotensin II from rat adrenal glands 24 hours post-nephrectomy. Mendelssohn could find no AII in the plasma. He suggested that the adrenal AII either had been concentrated from the plasma or synthesized in the adrenal gland. Naruse and Inagami, in 1982, [10] presented further evidence that the adrenal renin-like enzyme was a locally produced enzyme. Renin activity from the adrenal extracts was inhibited by a highly specific antibody to kidney renin. They also found that adrenal renin concentration in the spontaneously hypertensive rat (SHR) was higher than that of the Wistar-Kyoto rat (WKY) control rats. Nephrectomy markedly increased the concentration of adrenal renin in the SHR rat. The increase in renin many hours after nephrectomy made it unlikely that adrenal renin was derived from plasma renin. The half-life of plasma renin is about 10–20 minutes; thus there is virtually no circulating renin a few hours after nephrectomy, yet the adrenal renin concentration continues to rise. These authors have also reported renin in human adrenal cortex removed at surgery [11]; this human adrenal renin had many of the biochemical features of kidney renin. Our group [11,12] demonstrated that about 90 percent of the renin activity in the adrenal gland of the rat is localized in the outer zona glomerulosa cells of the adrenal gland. We also noted that renin concentration was stimulated by a low-sodium diet and decreased by a high-sodium diet. A high-potassium diet also increased adrenal renin concentration. Nephrectomy led to a marked increase by 20 hours, even though plasma renin levels were undetectable. None of these physiological maneuvers altered the small amount of adrenal renin in the inner zones (the fasciculata-medullary zones).

Renin has also been localized to the zona glomerulosa cells of the adrenal by the immunohistochemical studies of Deschepper et al. in 1986 [13]; renin seemed to be located in the steroid-secreting cells. It still remains a possibility that renin was merely being accumulated from the plasma and not synthesized within the cells. By three separate techniques, however, it has been clearly demonstrated that renin is synthesized in the cells. *(1)* Nephrectomy lowered plasma renin to undetectable levels within

a few hours, yet the adrenal renin concentration continued to increase, reaching a peak concentration between 24 and 36 hours after nephrectomy [10,12]; *(2)* renin messenger ribonucleic acid (RNA) has been located in the adrenals of the mouse [14] and of the rat by in situ hybridization [13] and by Northern blot and dot blot analysis [15]; *(3)* the production of renin by adrenal cells in culture. Our group developed an explant culture of adrenal capsular tissue, and they were grown in serum-free media for up to 48 hours. There was net production of pro-renin, renin, aldosterone, and angiotensin II by the explants [16]. The evidence, therefore, indicates that renin can be synthesized by the adrenal zona glomerulosa cells.

The exact biochemical nature of renin in different tissues has not been fully determined. While the renin gene is expressed in many tissues, the renins in the pituitary gland, renal cortex, and testes are immunologically distinct, as demonstrated by experiments employing different renin antibodies [13]. Katz and Malvin [17] have shown that renin in the plasma and in the kidney appears in six different forms that could be separated by isoelectric focusing. It is possible that renin undergoes different post-translational modifications in different tissues. If renin is glycosolated differently, the local renins may display different immunoreactive properties as well as different isoelectric profiles.

Other components of the renin-angiotensin system have been detected in the rat adrenal. Angiotensinogen messenger RNA has been found by Northern blot analysis [18]. There is, however, concern that these results might have been contaminated by angiotensin messenger RNA from the Brown fat cells in the adrenal capsule. Angiotensin converting enzyme has been found in the outer region of the adrenal cortex [19], but it is not certain in which cells the enzyme is located. Angiotensin I, II, and III can be measured in the rat adrenal and chromatographed with synthetic peptides on HPLC [20]. Over 70 percent of the AII immunoactivity in the rat adrenal glands concentrate in the zona glomerulosa, and the administration of the angiotensin I converting enzyme inhibitor, Captopril, for one week to rats caused a significant increase in adrenal AI and a decrease in AII and AIII. The concentrations of AI and AII in the adrenal cortex are much greater than that accounted for by contamination with blood and extracellular fluid.

## SUBCELLULAR DISTRIBUTION OF ADRENAL RENIN

The evidence appears quite clear, at least in the rat, that renin is located in the outer zone of the adrenal gland, primarily in the zona glomerulosa. Mizuno et al. [21] examined the subcellular distribution of adrenal renin by differential centrifugation of homogenates of adrenal capsules; they found renin associated with mitochondrial fractions. Our group has also found renin in this subcellular fraction [22]. Furthermore, by Percoll density gradient centrifugation, Mizuno et al. [21] noted that these dense granules were separated from mitochondria and microsomes. The number of dense granules increased after nephrectomy. Immunogold staining showed the presence of renin in these granules. It appeared, therefore, that the adrenal gland indeed does store a renin-like enzyme in granules. This storage of granules suggested it may be released into the extracellular space like other proteins that are stored in granules.

## REGULATION OF ADRENAL RENIN CONCENTRATION

Changes in sodium diet in rats alter the renin concentration in adrenal capsules. A low-sodium diet markedly increased adrenal renin concentration, while a high-sodium diet decreased it. There were no effects of changes in sodium intake on the small

amount of renin found in the decapsular portion of the adrenal. A high-potassium diet increased adrenal capsular renin, while at the same time plasma renin decreased; this result makes it unlikely that the increase in capsular renin was derived from the plasma. Previous studies by Naruse and Inagami [10] have shown that nephrectomy in the SHR rat increased adrenal renin. We confirmed their finding in the Sprague-Dawley rat and demonstrated a marked increase in adrenal capsular renin after nephrectomy. This increment occurred only in the capsular zone, with no change in the small amount of renin present in the decapsular zone containing the fasciculata-medullary portion of the adrenal gland [12]. Several mechanisms seem to play a role in the response to nephrectomy [23]. The pituitary gland, in a permissive way through ACTH release, is necessary for the maintenance of growth and function of the rat adrenal. The elevation of serum potassium is also important and by preventing the rise in serum potassium in the nephrectomized rats with the use of cation exchange resins, the elevation in the adrenal renin can be prevented. Finally, the lack of a negative feedback by circulating AII appears to be important. Bilateral nephrectomized rats infused continuously with synthetic angiotensin II did not show a rise in adrenal renin in response to nephrectomy, despite the fact that potassium levels were unchanged by the AII infusion, and plasma corticosterone values were slightly increased, suggesting further release of ACTH. Therefore, it appears that angiotensin II can have a negative feedback on production of adrenal renin, as it does in the kidney. It is also possible, however, that the angiotensin II was enhancing the release of renin from the adrenal glands. Mizuno et al. [21], in an *in vitro* explant culture, claimed an enhancement of renin release from these explants when angiotensin II was added to the media. They argued that the control of renin in the adrenal is different from that of the kidney. They also found increased release of renin by high potassium concentration in the media of the explants. This result is in contrast to what we find with *in vivo* potassium loading in rats, in which the concentration of renin goes up in the adrenal [11,12]. Also, in our own studies, using adrenal explants in more prolonged culture, we do not find increased release of renin into the media with increased potassium concentrations.

Genetic factors may also play a role since the spontaneous hypertensive rat strain (SHR) has a higher adrenal renin concentration than the Wistar-Kyoto (WKY) control. Also, we have shown that the Dahl salt-sensitive S rat has a lower adrenal renin concentration than the salt-resistant R rat. (Cross-breeding experiments strongly indicate these are genetic phenomena [24].) The Dahl S rat develops severe hypertension when placed on a high-sodium diet, while the R rat does not.

## PHYSIOLOGICAL FUNCTION OF ADRENAL RENIN

The fact that all the components of the renin-angiotensin system, including the active component angiotensin II, are generated locally in the adrenal gland suggests that the system has a local function. It is certainly true that other enzymes such as tonin, cathepsin G, kallikrein, and trypsin can produce AII directly from substrate without the intermediate AI or converting enzymes, but it would seem more likely that angiotensin II is produced through the renin system. It is also not clear whether all the components of the renin system are within a given cell, or whether the renin is secreted into the local circulation where it acts upon substrate in blood, resulting in AI which is then converted to AII. The locally generated AII may act locally or possibly at a more distant site.

Despite the presence of renin in a variety of tissues, no specific physiological role has

been clearly demonstrated. The renin-angiotensin system in vascular tissue has been postulated to play a role as a local vasoconstrictor. The renin-angiotensin system may have actions on tissues other than the classical endocrine function. Angiotensin II has been reported to be a potent mitogen for bovine adrenal cells [25]; it can stimulate the rate of proliferation and thymidine incorporation into cells [25]. AII may act within cells and has been shown in 1984 by Re and Parab to increase RNA synthesis by isolated hepatic nuclei *in vitro* [26]. High affinity receptors for AII have been identified in nuclear chromatin, suggesting a role for AII in enhancing transcription of specific genes [27]. The fact that renin is found in the zona glomerulosa cells, which also synthesize aldosterone, and the fact that the kidney renin-angiotensin system regulates aldosterone production suggest, however, the attractive hypothesis that adrenal renin is playing a role in the regulation of aldosterone.

Nevertheless, renin-angiotensin could be playing other roles. AII could be generated locally in the adrenal cortex and pass through the sinusoids into the medulla to regulate catecholamine excretion. It is known that angiotensin II can increase release of catecholamines from the adrenal medulla. It is also possible that the adrenal could be a source of renal-like activity found in the plasma of nephrectomized humans and animals. Converting enzyme can cause a hypotensive response in sodium-depleted anephric subjects [28], which suggests that renin and renin-like enzymes synthesized in extra-renal tissues may contribute to maintaining the blood pressure of anephric subjects. Adrenal tumors may secrete renin into the circulation. A patient with hypertension, hyperreninemia, and hyperaldosteronism was found to have a tumor that produced aldosterone and renin [5]. Removal of the tumor corrected the hypertension, hyperreninemia, and hyperaldosteronism.

The most logical concept is that the adrenal renin-angiotensin system is playing a role in regulating aldosterone. There is evidence to support this concept; however, most of it is indirect. In the studies of Doi et al. [11,12] in which they showed changes in sodium intake altered adrenal renin concentration, aldosterone concentration changed in the same direction; that is, a low-sodium diet, as expected, increased aldosterone concentration in the rat adrenal as well as adrenal renin, while a high-sodium diet decreased both. The high-potassium diet also increased adrenal renin concentration and, as expected, increased adrenal aldosterone concentration. After nephrectomy, aldosterone concentration was significantly increased along with the adrenal renin.

Thus, there is a strong positive correlation between adrenal renin and adrenal aldosterone concentrations. A further correlative relationship was seen in a Dahl salt-sensitive and a salt-resistant rat. The Dahl salt-sensitive rats are known to have low plasma renin and low plasma aldosterone. Adrenal renin and adrenal aldosterone concentration were considerably lower at both six and 14 weeks of age, compared with the salt-resistant rats [29]. Also, the response of adrenal renin to nephrectomy is diminished in the S rats. Furthermore, when S rats were placed on a sodium-deficient diet for one to two weeks, adrenal renin concentration responded much less than did the adrenal concentration in R rats. It would appear, therefore, that the low adrenal renin is associated with low plasma aldosterone and is not due to volume expansion, since sodium depletion could not correct this deficiency. The report by Nakamura et al. [20] suggested that the adrenal renin and angiotensin system is necessary for the proper aldosterone response to potassium stimulation. These authors administered Captopril to rats for several days and then removed the adrenals and *in vitro* showed that potassium stimulation of aldosterone was suppressed. This finding was correlated with

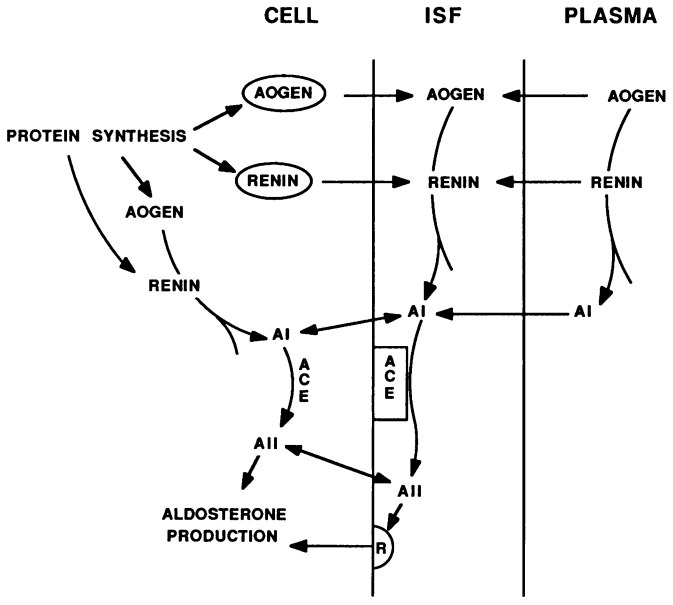


FIG. 1. Possible mechanisms for local and extra-adrenal control of aldosterone production. This cartoon depicts possible mechanisms of action of a local renin-angiotensin system as discussed in the text. **AOGEN,** angiotensinogen; **AI,** angiotensin I; **AII,** angiotensin II; **ACE,** angiotensin converting enzyme; **R,** receptor site for AII; **ISF,** interstitial fluid.

a low angiotensin II content of the adrenal gland. With a different approach, we confirmed these results. We grew adrenal capsular explants in tissue culture, and showed that they were consistently stimulated by an increase in potassium concentration in a culture media from 4–6 mm/L. The addition of a converting enzyme inhibitor, Lisinopril, to the culture media reduced both aldosterone and angiotensin II production in the presence of normal or high potassium concentrations [16].

These data, therefore, suggest a possible role of adrenal renin in the regulation of aldosterone. Whether this role is an important or trivial mechanism remains to be determined. Furthermore, it is not known whether all the components of the renin-angiotensin system are in the same cell; for example, in the brain, renin is in one area, while angiotensinogen is in other cells. The precise mechanism by which the adrenal renin-angiotensin system can alter aldosterone is also unknown. Adrenal renin could act in an autocrine or paracrine fashion. In the first possibility, most or all of the steps would occur within the cell, and little AII would be released from the tissue. Another possibility is that AII is formed intracellularly and released to bind AII receptors on adjacent cells. A third possibility is that renin and/or AI are released and further steps occur on the blood side of the cell membrane. For example, renin would be secreted into the sinusoidal blood to act on substrate in the blood to form AI. Then the converting enzyme on the surface of the endothelial cell membrane could convert AI to AII. AII would then bind to the angiotensin II receptor on the zona glomerulosa cell, leading to increased aldosterone production (Fig. 1).

In summary, the different components of the renin-angiotensin system have been identified in the adrenal cortex and appear to be synthesized there, at least in part. Prorenin, renin, converting enzyme, and angiotensin I and II have all been found primarily in the adrenal capsular cells. The messenger RNA for angiotensinogen and for renin have been reported to be present in the zona glomerulosa cells. It appears that renin may also be stored as granules in these cells and may at times be released into the circulation. The expression of the adrenal gene appears to be under physiological

control. There is a positive correlation between adrenal renin concentration and adrenal aldosterone concentration, and there is a suggestion that adrenal renin may be playing some role in the regulation of aldosterone production. Further studies are in progress to support or refute this hypothesis.

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