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Potassium-Stimulated Angiotensin Release from Superfused Adrenal Capsules and Enzymatically Dispersed Cells of the Zona Glomerulosa*

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ABSTRACT. The cells of the adrenal cortex contain angiotensin-II (AII), but whether this peptide is synthesized there (*vs.* internalized from the systemic circulation), whether it is secreted, and whether it is important in aldosterone production remain uncertain. To address these issues, we studied AI and AII release from superfused rat adrenal capsules and dispersed glomerulosa cells. Superfused adrenal capsules released 7-fold more AII in 270 min than the capsules originally contained (495 ± 101 fmol AII/rat released *vs.* 66 ± 8 fmol AII/rat tissue content). The amount of AI released in the same period only slightly exceeded the tissue content. In response to higher potassium concentrations in the medium (9 *vs.* 3.6 mM K^+), adrenal capsules and dispersed glomerulosa cells both released significantly more AI and AII into the superfusate. This release of AI and AII was oscillatory. The oscillations occurred in each of 15 experiments, with a period of 45–90 min. Decapsulated adrenal glands (the zona faciculata/reticularis plus medulla) also contained and released AII, but did not respond to potassium stimulation. There was a highly significant correlation between AII and aldosterone release. This was especially apparent if aldosterone secretion was examined during oscillations of AII release ($r = 0.97$; $P < 0.0001$). We conclude that AII is synthesized in the zona glomerulosa and can be released in response to stimuli. The close correlation between AII and aldosterone secretion suggests that locally produced AII may play an important role in aldosterone biosynthesis. (*Endocrinology* **129:** 823–831, 1991)

OVER the past 10 yr, our concept of the renin-angiotensin system (RAS) has changed dramatically. The original concept was that of a circulation-based hormonal system which generated angiotensin-I (AI) and angiotensin-II (AII) in the plasma. However, the demonstration that most of the target tissues of AII contain not only AII receptors but also all of the components necessary for AII generation has broadened our understanding of the RAS (1–10) and gradually challenged the classic concept (11–17). As this list of tissues that respond to AII grew, it became increasingly difficult to explain how such widespread tissues with such diverse actions could all be controlled by the plasma AII level, which bathes all tissues uniformly. The existence of AII generation within these target tissues provides the potential for each tissue to regulate its RAS via different stimuli (*e.g.* neurotransmitters, pituitary hormones, volume status, *etc.*) and, thus, allows the local RAS to function in a precise, tissue-specific fashion. These considerations support the concept that local AII production in various tissues may be more important than the circulating level.

The adrenal cortex is a good example of this conceptual evolution. Aldosterone production has been presumed to be regulated by the circulating RAS. However, all of the components of the RAS have been described in the adrenal (18). In this study we set out to answer three questions. First, is adrenal cortical AII produced locally or, as has been argued by some past studies, is it merely the result of receptor-mediated internalization of circulating peptide (19, 20)? Second, is AII secreted from glomerulosa cells and is that secretion regulated by, for example, potassium? Third, does locally produced AII play a role in aldosterone secretion?

Materials and Methods

Preparation of zona glomerulosa tissue slices

The adrenal glands of 10 female Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA), weighing 200 g, were used for each experiment. After decapitation, the adrenal glands were removed, and the capsules (zona glomerulosa) were separated from the fasciculata/medulla on an ice-cold support using the standard method of our laboratory (21). The capsules were separated into two equal pools and washed with

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ice-cold medium 199 containing 3.6 mM K^+, 25 mM sodium bicarbonate, and 0.1% tissue culture quality BSA (Sigma, St. Louis, MO) and saturated with a mixture of 95% O_2 and 5% CO_2. After this, the capsules were kept in a fresh aliquot of ice-cold medium until they were loaded in the superfusion chamber.

Preparation of dispersed zona glomerulosa cells

Adrenal capsules obtained from 20 female Sprague-Dawley rats were collagenase dispersed using the standard procedure of our laboratory (21). The resulting cells were washed with medium 199, then mixed with preswollen Sephadex G-15 and loaded into 4 superfusion chambers, usually 2–6 × 10^6 cells/chamber.

Superfusion system

For this purpose we have employed the superfusion system of Forma Scientific (Forma Scientific, Marietta, OH), modified by increasing the diameter of the 0.5-ml superfusion chambers to 9 mm. The capsular tissue from 10 rats was divided into 2 equal pools. Groups of 5 capsules were chopped in pieces less than 1 mm in diameter with an Olfa rotary cutter (Olfa Corp., Osaka, Japan). The chopped tissue was mixed with Sephadex G-15 (which had been boiled in water and equilibrated in medium 199) and loaded into 4 superfusion chambers atop a Sephadex G-15 layer and a 5-μm nylon filter. The superfusion was started as soon as possible. For the first 90 min all four chambers were superfused with medium 199 containing 3.6 mM K^+ (37 C), with a flow rate of 0.2 ml/min·chamber. After this period, in the following 180 min (experimental period), 2 chambers continued to be superfused with the same medium, and the other 2 chambers were perfused with the same medium but containing 9 mM K^+. This design made it possible to compare the effects of high and low potassium levels on the adrenal capsules of the same animals. For experiments using dispersed cells of glomerulosa tissue, the 4 chambers were loaded with cells from the same collagenase digestion, assuring comparable populations in each chamber.

After the perfusate exited the chamber, an equal volume of inhibitor solution was mixed with the superfusate, using a micro-T-joint. The final concentrations of these inhibitors were: EDTA, 5 mM; phenylmethylsulfonylfluoride, 200 micromolar; and captopril, 200 μM.

The superfusate was collected separately for each channel in 5-min aliquots in polyethylene tubes containing 0.1 ml 50% glycerol dissolved in 10% phosphoric acid, and was quickly frozen in a mixture of dry ice and ethanol. For analysis we pooled 3 such fractions, yielding 6 pairs of 15-min fractions for the first 90 min and 12 pairs for the main experimental period of 180 min. All of these samples were lyophilized.

Tissue angiotensin content and HPLC separation of peptides

The methods of tissue processing, extraction, and HPLC separation of angiotensin peptides have been described previously (22). In brief, the separated zona glomerulosa was frozen in liquid nitrogen, homogenized with 8 M urea containing 0.1% Triton X-100, and extracted with 80% methanol containing 10 mM sodium acetate and 0.5% trifluoroacetic acid. The angiotensin content of this extract was measured by RIA after HPLC separation. To establish the nature and proportions of different angiotensin peptides responsible for AI and AII immunoreactivity of the superfusate, in a limited number of experiments (n = 2) two to four superfusion fractions were pooled, and the angiotensins were separated by HPLC and quantitated by RIA.

RIA

We have used a double antibody method for AI and AII RIA. The first antibody against AI or AII (Arnel, New York, NY) cross-reacted 100% with the Des-Asp-AI and Des-Asp-AII, respectively. The anti-AII antibody also had a 0.1% cross-reactivity with AI. [This is not important when the amount of peptides was determined after HPLC separation. However, when HPLC was not employed, *e.g.* in crude extracts or in the superfusate, which was not always chromatographed, we use the terms immunoreactive AI (AI_{ir}) and immunoreactive AII (AII_{ir}).] The second antibody, a donkey antirabbit immunoglobulin, was bound to a magnetic gel carrier (Amerlex M separation reagent, Amersham, Arlington Heights, IL). The [^{125}I]AI and [^{125}I]AII were purchased from New England Nuclear (Boston, MA). In superfusion experiments, an aliquot of medium 199 along with the inhibitor mixture was also lyophilized and redissolved in the same amount of water as the samples, and was used as an assay buffer for the RIA standard curves. The lyophilized samples, obtained during superfusion, were redissolved in 2 ml deionized water. The samples obtained after HPLC were dried in a vacuum concentrator and redissolved in 1 ml RIA assay buffer (22).

Duplicate samples of 0.4 ml for each AI, AI_{ir}, AII, or AII_{ir} were mixed with 100 μl [^{125}I]AI or [^{125}I]AII (5000 cpm/100 μl) and 100 μl corresponding first antibody. After 24 or 48 h, the bound/nonbound fractions were separated with the second antibody, bound to a magnetic gel carrier. The results were evaluated and computed in a Micromedic 400 γ-counter (Micromedic, Horsham, PA) connected to an IBM AT computer fitted with RIA-Aid software (RMA, Inc., Cambridge, MA). Because of the very low level of angiotensins in some samples obtained after HPLC (*e.g.* superfusates of tissue and cells), we developed a RIA with greater sensitivity for those samples. Each HPLC fraction was divided into two equal parts and dried in the presence of glycerol in a vacuum concentrator. Then, the thin film of glycerol containing the peptides was mixed with the tracer, and the first antibody was dissolved in a volume of 50 μl each and incubated at 4 C for 48 h. The counts per min of the tracer and the concentration of the first antibody were reduced by 40% and 75%, respectively, compared with those in the standard assay. The sensitivity of the assay increased considerably to 0.2 fmol AI/sample and 0.25 fmol AII/sample. The coefficients of variation at 8 and 32 fmol were less than 5%. However, the upper limit of the assay dropped to 64 fmol/sample. The reproducibility of the results was increased by the use of a Gilson 212B liquid handler (Gilson, Middleton, WI) connected to an IBM PC.

Aldosterone was assayed using a commercial RIA kit (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA).

Statistics

Data are displayed as the mean ± SEM. The significance of differences observed between released angiotensin and tissue

content and that between angiotensin secretion of tissue slices superfused with medium containing 3.6 and 9.0 mM K^+ were calculated with the Mann-Whitney test. The oscillatory release of angiotensins was tested with the mean square successive difference test (23). The correlations and regression lines were calculated and plotted using the Sigmaplot software (Jandel Scientific, Sausalito, CA).

Results

Does the zona glomerulosa produce angiotensins?

Zona glomerulosa tissue slices superfused with medium 199 containing 3.6 mM K^+ released 495 ± 101 fmol AII_{ir}/rat (n = 4) into the medium in 270 min. The tissue content of AII, measured after separation of angiotensins by HPLC in a different group of animals, was 66 ± 8 fmol AII/rat (n = 4). Thus, superfused zona glomerulosa tissue released 7.4 times more AII_{ir} in 270 min than the mean AII content of the tissue before superfusion (Fig. 1).

The pattern of AI_{ir} release was different from that of AII_{ir} release. The amount of AI_{ir} released (112 ± 2 fmol AI_{ir}/rat; n = 4) in the same time period from the same tissue as AII_{ir} was similar to the average initial tissue content (79 ± 30 fmol AII/rat; Fig. 1).

The molar ratio of AII_{ir} to AI_{ir} was 4.7 in the superfusate. However, the ratio of AII to AI in the tissue was 2.2 before and 2.3 after superfusion, indicating that AII is the preferred secretory product.

To circumvent the possibility that our superfusate RIA measurements were measuring other cross-reacting peptide fragments as well as AI and AII, we separated the superfusate peptides by HPLC before RIA in two experiments. While the capsules themselves contained appreciable amounts of Des-Asp-AI and Des-Asp-AII, the superfusate contained only AI and AII (Fig. 2). In addition, in one experiment using 20 rats, we extracted 20 capsules, superfused the other 20 capsules, and then

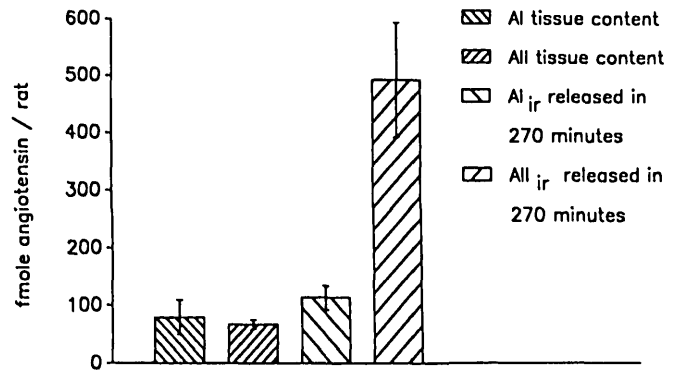

FIG. 1. Superfused zona glomerulosa tissue slices released (in 270 min) 7-fold more AII_{ir} than the original AII content in the tissue ($P < 0.01$). The AI_{ir} released at the same time from the same tissue was similar to the tissue content. The values represent the mean ± SEM of four independent experiments.

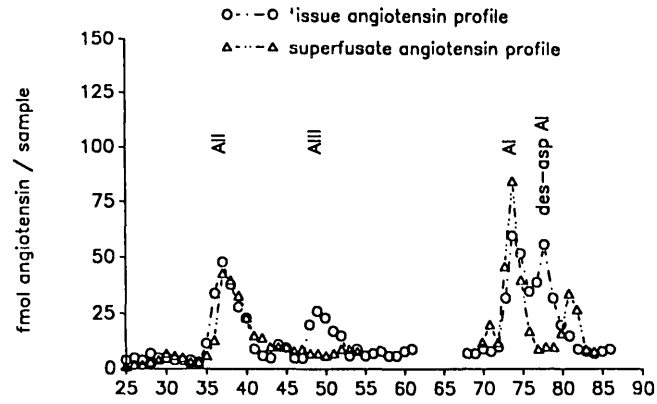

FIG. 2. HPLC separation of angiotensin peptides from adrenal capsular slices and superfusate. Each HPLC fraction (numbered on *abscissa*) was assayed using immunoassays directed against AII (fractions 25–61) or AI (fractions 68–86). The angiotensin peptide profiles of tissue slices and the superfusate are different. The tissue contains not only AI and AII, but also Des-Asp-AI and Des-Asp-AII (AIII). However, the superfused tissue secretes mainly AII and AI. No significant amounts of AIII or Des-Asp-AI were detected in the superfusate. The small peak of AI immunoreactivity at superfusate fraction 82 does not comigrate with any angiotensin fragment we have tested and remains unidentified.

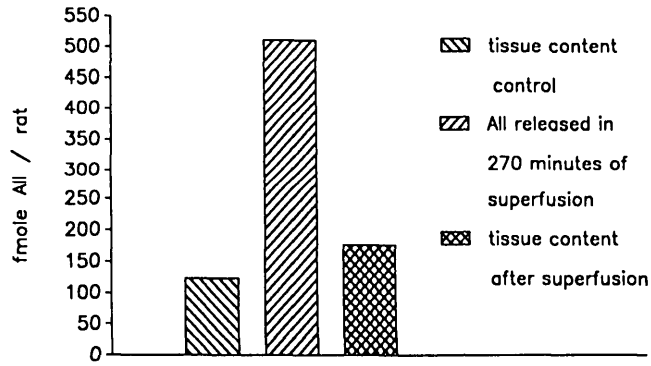

FIG. 3. The same pool of zona glomerulosa tissue obtained from 20 rats was divided; 1 part was extracted, and the other was superfused then extracted. All of the extracts and the superfusate were chromatographed (HPLC), then the angiotensin content was measured by RIA. The amount of AII released in 270 min was more than 4 times the initial tissue content. In addition, the tissue content after superfusion slightly exceeded the initial level.

extracted their remaining angiotensin peptides after superfusion. All of the tissue extracts and superfusates from this experiment were subjected to HPLC, and the separated angiotensins were quantitated by RIA. The amount of AII released into the medium during a superfusion period of 270 min was 511 fmol AII/rat. The control tissue contained 123 fmol AII/rat. After superfusion, the tissue content was 176 fmol AII/rat (Fig. 3). Thus, the total amount of AII released during superfusion was 4-fold more than the initial tissue content, and the tissue content after superfusion was maintained despite this released peptide.

Is angiotensin release regulated?

The release of AII_{ir} from zona glomerulosa tissue slices declined steeply during the first 30 min of superfusion, and then more gradually over the next 240 min. In response to 9.0 mM potassium in the superfusate, tissue slices displayed an increase in AII_{ir} release within the first 15 min, and the release rate remained elevated over the entire 180-min period of superfusion compared to 3.6 mM K^+ (Fig. 4). We compared AII_{ir} release in response to 3.6 *vs.* 9.0 mM K^+ in 12 experimental periods of 15 min each in parallel chambers perfused with low *vs.* high potassium concentrations using the Mann-Whitney test. AII_{ir} was significantly greater during 9.0-mM K^+ superfusion ($P < 0.001$; n = 4).

During superfusion of dispersed glomerulosa cells, a similar initial decline in AII_{ir} release was seen, but with 3.6 mM K^+ in the medium, the basal level of AII_{ir} release remained stable over the next 180 min of observation. In response to 9.0 mM K^+, glomerulosa cells exhibited a greater initial burst of AII_{ir} release and then a persistent increase in AII_{ir} release over 180 min (see Fig. 5). As with tissue slices, the glomerulosa cells released significantly more AII_{ir} in response to 9.0 mM K^+ ($P < 0.001$).

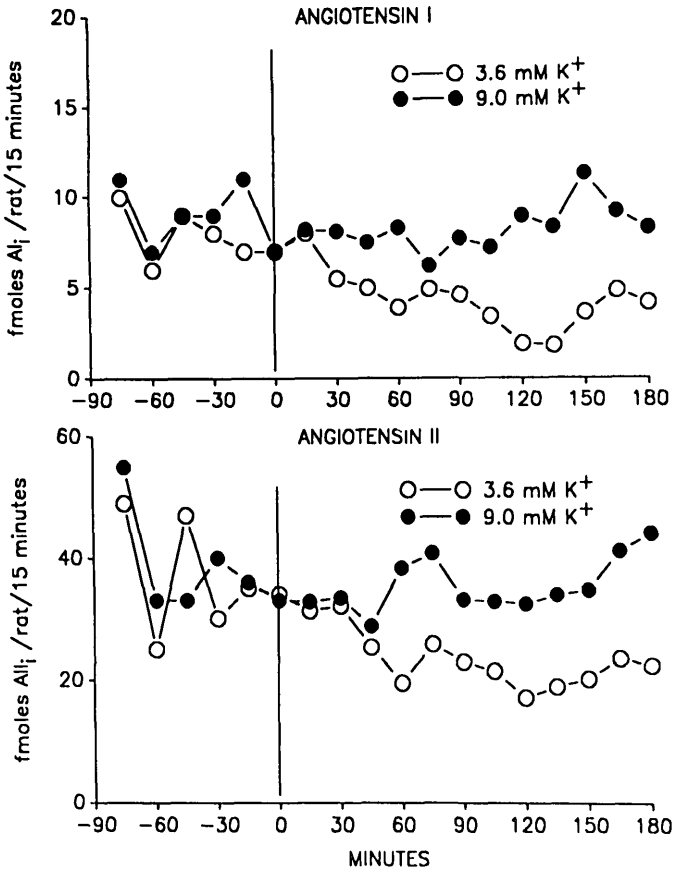

FIG. 4. The time course of AI_{ir} and AII_{ir} release from superfused zona glomerulosa tissue slices. An increase in the extracellular potassium concentration from 3.6 to 9 mM at time zero significantly increased ($P < 0.001$) AI_{ir} and AII_{ir} release (n = 4 experiments).

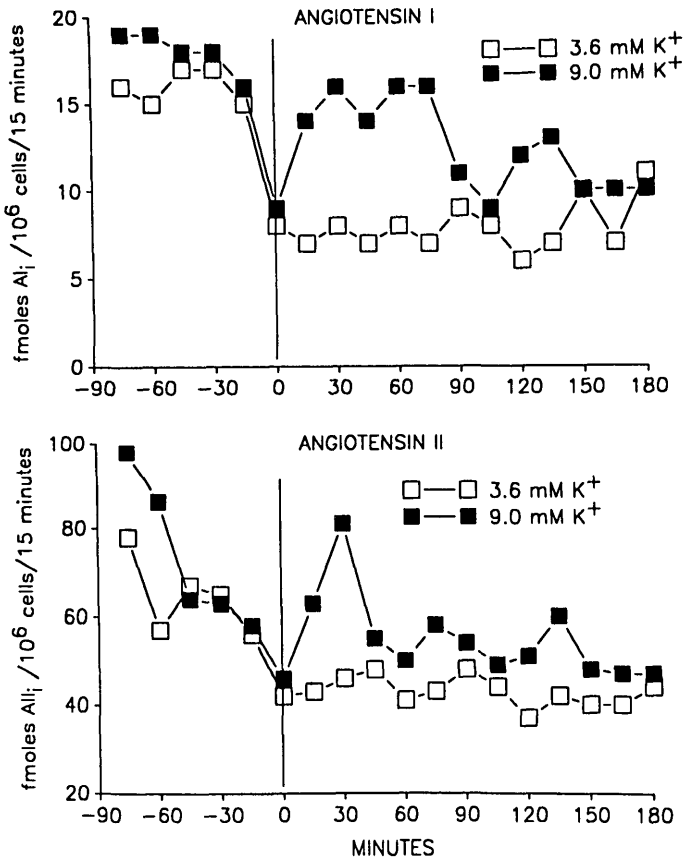

FIG. 5. The time course of AI_{ir} and AII_{ir} release from superfused enzymatically dispersed cells of zona glomerulosa tissue. Potassium (9 mM) administration, initiated at time zero, sharply and significantly ($P < 0.001$) increased the release of angiotensins.

TABLE 1. The amounts of AI and AII release during 180 min of superfusion with 3.6 or 9 mM potassium

	AI_{ir}	AII_{ir}	AII_{ir}/AI_{ir} ratio
Dispersed glomerulosa cells (fmol peptide/10^6 cells)			
3.6 mM potassium	101 ± 35.7	522 ± 158	5.2
9.0 mM potassium	151 ± 55.2	646 ± 193	4.2
Adrenal capsular tissue (fmol peptide/rat)			
3.6 mM potassium	49.5 ± 27.3	280.3 ± 38.4	5.7
9.0 mM potassium	83.2 ± 25.7	426.6 ± 90.8	5.1

Results were obtained in four independent experiments.

The pattern of AI_{ir} release parallelled that of AII_{ir}. After an initial decline, there was a burst of AI_{ir} in response to 9.0 mM K^+. However, the absolute amounts of AI_{ir} were consistently less than those of AII_{ir} (Figs. 4 and 5). The cumulative values of angiotensin release in 180 min by dispersed cells or tissue slices after superfusion with 3.6 and 9 mM K^+ are shown in Table 1. Since 9 mM K^+ caused a relatively greater increase in AI_{ir} than in AII_{ir} release, the ratio of AII_{ir} to AI_{ir} was consistently smaller in the medium containing 9 mM K^+ (Table 1).

In contrast, the superfused decapsulated gland containing medulla and zona fasciculata released more AII_{ir} (on a per rat basis) than did capsular tissue. However,

neither AII_{ir} nor AI_{ir} release was affected by increasing the K^+ content of the medium. In three experiments, the decapsulated glands released 471 ± 96 fmol AII_{ir}/rat·180 min with 3.6 mM K^+ in the medium and 490 ± 77 fmol with 9 mM K^+. AI_{ir} release (in one experiment) was 93 fmol/rat·180 min with 3.6 mM K^+ and 100 fmol with 9 mM K^+. In two other experiments, AI_{ir} release was too low to be detected with our assays.

Oscillatory pattern of angiotensin release

Angiotensin release from both cells and tissue slices displayed a wave-like oscillatory pattern. We define an oscillation not as an increase in peptide release in a single 15-min period, but, rather, as a consistent increase and then a gradual decrease in release over two to four sequential 15-min measurements. The relative amplitudes of oscillations were the same for AI_{ir} and AII_{ir}. Since averaging the peptide values from several experiments tends to obscure the oscillatory pattern of angiotensin release, in Fig. 6 we present the results of an individual experiment.

We tested the sequential randomness of AI_{ir} and AII_{ir} values in the channel superfused with 3.6 mM K^+. The mean square successive difference test was applied in 15 independent experiments with both tissue slices and dispersed cells, using 18–22 consecutive sampling periods/experiment. In every experiment our analysis indicated that these oscillations were not random events ($P < 0.0005$ in 8 cases, $P < 0.0025$ in 3 cases, $P < 0.01$ in 1 case, and $P < 0.025$ in 3 cases).

AI_{ir} and AII_{ir} release displayed parallel increases or decreases (Fig. 6). There was a significant correlation ($P < 0.001$) between the amounts of AI_{ir} and AII_{ir} released into the medium in each 15-min period with both 3.6 and 9 mM K^+. Figure 7 displays this correlation for capsular tissue. A similar correlation existed with superfused dispersed cells (r = 0.71; $P < 0.001$; n = 51 pairs).

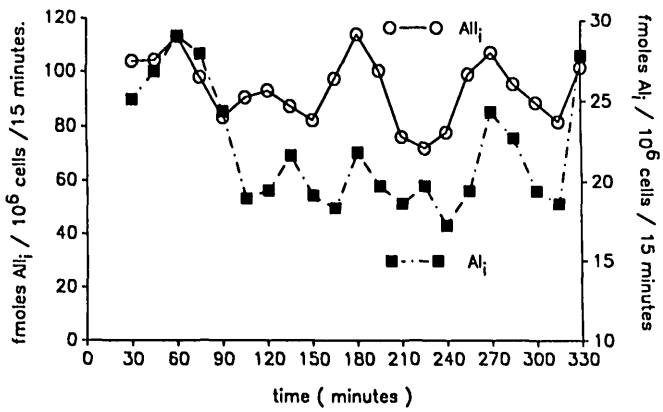

FIG. 6. Angiotensins were released in an oscillatory wave-like pattern. The sampling period of 15 min does not permit detection of sharper secretory peaks. The probability of random generation of this secretory pattern, tested with the mean square successive difference test, was $P < 0.025$ in each of 15 independent experiments.

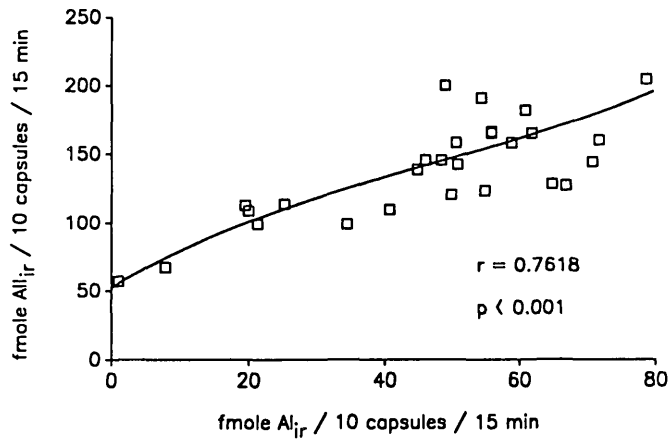

FIG. 7. There was a significant correlation ($P < 0.001$) between the amounts of AI_{ir} and AII_{ir} released from superfused tissue slices into the superfusate, suggesting a common origin and a synchronous release of AI_{ir} and AII_{ir}.

Does local AII release regulate aldosterone production?

Increasing the potassium concentration in the superfusion medium was followed by increased aldosterone secretion from both tissue slices and dispersed cells. Cells superfused with 3.6 mM K^+ released 0.26 ± 0.6 ng aldosterone/10^6 cells·15 min (n = 4). After stimulation with 9 mM K^+, the same cells released 20.6 ± 11 ng aldosterone/15 min (four independent experiments). Superfused tissue slices displayed similar aldosterone release.

The pattern of aldosterone release after stimulation with high potassium was similar to that of AII release. The initial high peak was followed by a sustained aldosterone release with decreasing levels and oscillations, documenting that aldosterone release parallelled AII release. Indeed, when superfused with 3.6 and then 9 mM K^+, there was a highly significant correlation between the amount of AII_{ir} and the logarithm of the aldosterone secreted by both the zona glomerulosa tissue slices (r = 0.76; $P < 0.001$; data not shown) and enzymatically dispersed cells (r = 0.83; $P < 0.001$; n = 45). However, the correlation coefficient indicated a much better fit if the correlation was calculated (using the same data) between the amount of AII_{ir} secreted during a secretory oscillation or cycle and the amount of aldosterone secreted during the same period of time (Fig. 8).

Discussion

Zona glomerulosa contains not only AII in a concentration that exceeds the plasma level by 100-fold, but it also contains all of the necessary components of the enzymatic cascade to generate AII. Both AII content (22) and the expression of renin and angiotensinogen genes (24, 25) as well as renin activity (26) are regulated by salt intake. However, the origin of the intracellular AII content and the site of AII production (intracellular *vs.*

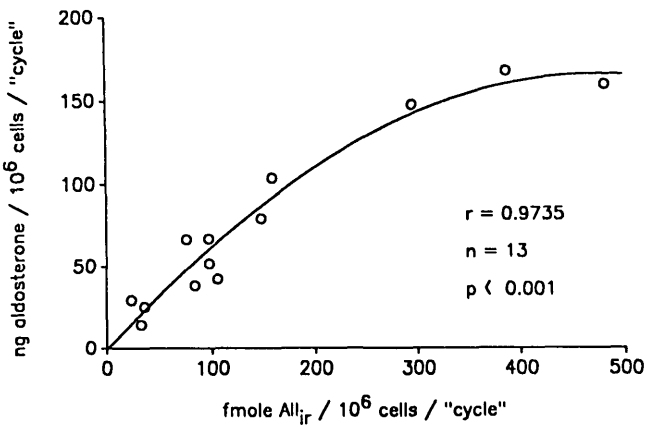

FIG. 8. There was a highly significant ($P < 0.001$) correlation between the amount of AII_{ir} and aldosterone released during secretory periods.

extracellular) are a matter of debate (19, 20).

Our results clearly show that superfused capsular tissue is capable of net synthesis of AI and AII. During 270 min of superfusion with 3.6 mM K^+, the zona glomerulosa releases several-fold more AII into the medium than was present in tissue at the beginning of the superfusion. Since the tissue peptide content at the end of superfusion is practically unchanged from its starting value, all of the AII released during this period must have been synthesized *de novo*. Our results confirm previous observations using bovine zona glomerulosa (27) or rat adrenal capsules (28) grown in tissue culture for 24 h. These studies have also noted net production of AII. However, the rate of accumulation of AII in a static medium begins to decline even after 60 min (3); therefore, quantitative assessment of angiotensin production during a 24-h static incubation is hampered by factors such as peptide degradation, uptake, and possible feedback inhibition of secretory activity. In addition, assaying the angiotensin content of the medium after a static incubation gives no information about the time course of angiotensin release in basal conditions or after stimulation. Thus, our study also extends those previous findings by demonstrating oscillatory secretion of AI and AII that is rapidly responsive to potassium stimulation.

In contrast to that of AII, the amount of AI or AI_{ir} released during the same 270-min period only slightly exceeds the initial tissue content of AI. The AII/AI ratio is, therefore, higher in the superfusate than in the tissue. A similar pattern of AII and AI release has been found in cells from a murine Leydig cell tumor. These cells release 90% of their AII content into the medium, but only 15% of AI and 1–2% of renin activity are released into the culture medium (29).

This secretory pattern of AII and AI supports not only the notion of local AII production, but offers an insight into the possible angiotensin-processing mechanism inside the cells. In peptide-secreting cells, the enzymatic conversion of the substrate and its molecular segregation occur in the Golgi stacks and in the *trans*-Golgi network (30, 31). This facilitates a gradual enrichment of the end product in the secretory granules. Consequently, these cells secrete mainly the end product, and in the final stage of the secretory pathway, one would not expect that all of the components of the peptide-producing cascade would be found in the final secretory granules. This lack of colocalization of renin and AII has been reported in zona glomerulosa (20). In addition, it is noteworthy that while the cells contain appreciable amounts of Des-Asp peptides (22), no Des-Asp products are released into the superfusate. This suggests that these peptides could represent intracellular degradation products of angiotensins that have been released and then reinternalized via cell surface receptors or perhaps even that have been shunted directly from secretory granules into lysosomes (crynophagy). At present, there are no data that allow us to speculate which of these possibilities is the most likely.

An increased concentration of extracellular potassium is a secretagogue for aldosterone (32, 33) as well as other peptide hormones (34). Superfusion with 9 mM K^+ increases angiotensin secretion from zona glomerulosa (both tissue slices and enzymatically dispersed cells). An initial burst of AI and AII release, evident during the first 15 min of high potassium superfusion, was followed by a sustained increase in release over the ensuing 180 min. The initial peak of secretion was more pronounced from dispersed cells and somewhat blunted using tissue slices. While the secretion of both peptides increases, the increase in AI secretion is relatively greater than that in AII during potassium stimulation. This change in the AII/AI ratio suggests a lower efficiency for the conversion of AI to AII. Perhaps, potassium stimulation increases the transit rate through the angiotensin secretory pathway and thereby decreases the efficiency of the AI to AII conversion. Indeed, potassium loading *in vivo* causes a conspicuous hypertrophy of the rat zona glomerulosa, primarily the endoplasmic reticulum, mitochondrial compartment, and Golgi network (35), *i.e.* the sites of intracellular peptide (and aldosterone) processing and targeting. In addition, *in vivo* potassium loading increases the AI and decreases the AII content of the adrenal gland (18), again suggesting that potassium interferes with the functions of this intracellular peptide secretory pathway.

The regulation of the angiotensin secretory pathway is different in the medulla and zona glomerulosa. Although decapsulated adrenal glands (inner cortical zones and medulla) release AI and AII during superfusion with medium containing 3.6 mM K^+, the increase in extracellular potassium to 9 mM had no influence on angiotensin secretion. Previous studies have also shown that renin

content as well as AI, AII, and AIII contents are not regulated by changes in salt intake in the adrenal medulla (22, 26).

Our superfusion system demonstrated that the release of AI, AII, and aldosterone is oscillatory. Many pulsatile secretory systems have been described in recent years. In some, the amplitude and/or frequency of secretory peaks are important as regulatory mechanisms (36–39). For example, the effect of ACTH on aldosterone production is lost if ACTH levels are tonically increased, but is sustained if ACTH is given in a pulsatile fashion (40). Thus, the pulsatile nature of angiotensin release by the zona glomerulosa may be further evidence that this release is a carefully regulated process. The basis for the pulsatility remains uncertain. The possibility that the released AII exerts an inhibitory feedback on its own further release is intriguing and could generate an oscillatory secretory pattern by itself. Protein kinase-C, activated by AII in zona glomerulosa cells, is important not only in the activation of cell functions, but also in feedback inhibitory actions, having a complex modulatory effect on Ca^{2+}-activated processes (41, 42). Other possibilities include regulation of synthesis, vesicular transport, or the secretory process itself.

Past studies have debated where adrenal AII is formed. Is it formed intracellularly, or is AI synthesized and secreted, then converted to AII by converting enzyme on nearby cells? The fact that dispersed glomerulosa cells, with the normal adrenal microarchitectural structure completely disrupted, secrete AII as efficiently as tissue slices suggests that AII is formed intracellularly and secreted as the mature peptide from these cells. This is in contrast to the brain renin-angiotensin system where products from at least two cell types may be needed to produce AII (9, 43, 44).

The close correlation we observed between AII and aldosterone release during superfusion suggests that local angiotensin release may play an important role in aldosterone secretion. This correlation was evident during both 3.6- and 9-mM K^+ stimulation, indicating that at both extracellular K^+ concentrations, aldosterone release correlates with AII release. Even more impressively, angiotensin and aldosterone seem to follow a similar pulsatility during stimulation with 9 mM K^+, and the closest correlation was found when we analyzed angiotensin and aldosterone release during these oscillatory cycles. While correlations such as these do not prove a cause and effect relationship, the fact that correlations existed during high and low K^+ superfusion and especially when analyzed as oscillations strongly suggests that a cause and effect relationship does exist. Other studies have suggested that AII is involved in aldosterone's response to potassium. Converting enzyme inhibitors or AII antagonists blunt the effect of potassium on aldosterone secretion both *in vivo* and *in vitro* (45–48). These data support the concept of autocrine and/or paracrine regulation of aldosterone secretion by locally produced AII and a connection among potassium, AII, and aldosterone.

We believe that the results of our current study coupled with previous reports help to clarify the nature, regulation, and functional importance of the local adrenal RAS. The fact that superfused tissue slices or enzymatically dispersed zona glomerulosa cells release, in a relatively short time, several-fold more AII than the initial tissue content proves that there is local production of AII. In addition, the fact that 10^6 cells release, under basal conditions, a mean of 40 fmol AII in 15 min and a mean of 80 fmol AII during stimulated peak secretory activity raises an important possibility. Since locally produced AII is released into a very small intercellular fluid volume (and with relatively slow fluid movement), the local concentration of AII in the intercellular space could be very high. This has already been shown in distinct fluid compartments of the kidney, where the AII concentration is 1000 times the plasma values (49). This means that the plasma level of AII is far less than the local AII concentration in some tissues and opens the possibility of very specific local tissue regulation.

This regulatory specificity offered by tissue RASs may be the key in understanding how AII modifies the function of diverse tissues. The factors that stimulate RAS activity are known to be different in these different tissues (6, 18, 22, 24, 25, 29, 50, 51). In addition, the cellular processes that AII controls are specific for each tissue and vary according to the AII concentration required as well as their timing (6, 15, 26, 27, 52–57) (*e.g.* during the ovulatory cycle). It is highly unlikely that such specialized responses are all regulated by the circulating AII level, which bathes all tissues similarly. This ability of different tissues to independently regulate local AII production provides the necessary specificity to control such diverse functions in such widely dispersed target cells.

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