Renal interstitial corticosterone and 11-dehydrocorticosterone in conscious rats

Kristie Usa,1 Ravinder J. Singh,2 Brian C. Netzel,2 Yong Liu,1 Hershel Raff,3,4 and Mingyu Liang1

1Department of Physiology, Medical College of Wisconsin, Milwaukee; 2Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota; 3Department of Medicine, Medical College of Wisconsin, Milwaukee; and 4Endocrine Research Laboratory, Aurora St. Luke’s Medical Center, Milwaukee, Wisconsin

Submitted 7 December 2006; accepted in final form 23 March 2007

Usa K, Singh RJ, Netzel BC, Liu Y, Raff H, Liang M. Renal interstitial corticosterone and 11-dehydrocorticosterone in conscious rats. Am J Physiol Renal Physiol 293: F186–F192, 2007. First published March 27, 2007; doi:10.1152/ajprenal.00484.2006.—Deficiencies in the conversion between active and inactive glucocorticoids in the kidney can lead to hypertension. However, the significance of glucocorticoid metabolism in specific kidney regions in vivo is not clear, possibly in part due to the difficulty in measuring glucocorticoid levels in kidney regions in vivo. We used microdialysis techniques to sample renal interstitial fluid from conscious rats. The levels of corticosterone (active) and 11-dehydrocorticosterone (inactive) were analyzed by liquid chromatography-tandem mass spectrometry. Direct infusion of the 11β-hydroxysteroid dehydrogenase (11β-HSD) inhibitor carbenoxolone into the renal medulla induced hypertension, and significantly increased corticosterone levels and the corticosterone/11-dehydrocorticosterone ratio, an index of 11β-HSD activity, in the renal medullary microdialysate, but not in urine or the plasma. Further characterization of conscious, untreated rats (n = 13–16) indicated that corticosterone concentrations (ng/ml) were 0.8 ± 0.1, 1.0 ± 0.1, 66.7 ± 8.1, and 7.9 ± 1.1 in cortical microdialysate, medullary microdialysate, the plasma, and urine, respectively. The corticosterone/11-dehydrocorticosterone ratios were 0.8 ± 0.1, 0.6 ± 0.1, 10.6 ± 1.4, and 1.7 ± 0.1, respectively, in these 4 types of sample. The expression level of 11β-HSD1 was higher in the medulla than in the cortex, whereas 11β-HSD2 was most enriched in the outer medulla. Microdialysate levels of corticosterone were ~1.6-fold higher in afternoons than in mornings, whereas plasma levels differed by 2.8-fold. These results demonstrated that corticosterone excess in the renal medulla might be sufficient to cause hypertension and provided the first characterization of renal interstitial glucocorticoids.

The biological activity of glucocorticoids in target organ systems is controlled not only by circulating levels of glucocorticoids but also by intracellular metabolism in target tissue. The metabolism of glucocorticoids in target tissue involves several enzymatic reactions including the interconversion between active and inactive glucocorticoids catalyzed by 11β-hydroxysteroid dehydrogenases (11β-HSD) (7, 41). The type 2 isozyme of the enzyme, 11β-HSD2, converts active glucocorticoids, mainly cortisol in human and corticosterone in rodents, to inactive forms, cortisone in human and 11-dehydrocorticosterone in rodents. The type 1 isozyme of the enzyme, 11β-HSD1, has both dehydrogenase and reductase activities but may act predominantly as a reductase in vivo, regenerating biologically active glucocorticoids from their inactive forms (40).

Local metabolism of glucocorticoids has particular relevance to renal function. Aldosterone is a powerful and important regulator of electrolyte transport in the distal nephron. Mineralocorticoid receptors in the distal nephron, however, can bind aldosterone and glucocorticoids with nearly equal affinity. 11β-HSD2 in the distal nephron inactivates glucocorticoids and allows aldosterone to be functionally significant (12, 35). Deficiencies in 11β-HSD2 can lead to the development of apparent mineralocorticoid excess syndrome (28, 36, 42) characterized by hypertension and hypokalemia due to overstimulation of mineralocorticoid receptors by excess glucocorticoids. Renal excess of glucocorticoids could also contribute to sodium retention and potassium loss in other diseases such as cirrhosis (11). The kidney also appears to express 11β-HSD1 (5, 31), but its significance is not known.

The kidney consists of anatomically and physiologically distinct regions including the cortex, the outer medulla, and the inner medulla. Differential expression of 11β-HSDs has been reported in the renal medulla of rat models of hypertension (23). 11β-HSD expression or activity in glomeruli, renal tubules, and vasculatures has also been reported (2, 3, 9). However, the significance of regional glucocorticoid metabolism in the kidney in vivo is not clear. This is possibly in part due to the difficulty in directly measuring glucocorticoid levels in kidney regions in vivo. Measurement of active and inactive glucocorticoids or their tetrahydro metabolites in urine is often used as an indirect index of renal metabolism of glucocorticoids. Renal interstitial microdialysis is an established technique used to obtain samples that can be used to estimate renal interstitial fluid concentration of a wide variety of substances such as cyclic GMP (33), ATP (30), nitric oxide metabolites (22), and catecholamines (1). Microdialysis has been used in the analysis of corticosterone in the brain (26), but not in the kidney. Microdialysis of 11-dehydrocorticosterone has not been reported.

In the present study, we examined the functional significance of regional metabolism of glucocorticoids in the kidney and tested the hypothesis that inhibition of 11β-HSD by carbenoxolone locally in the renal medulla would be sufficient to cause hypertension. Furthermore, we characterized the levels of corticosterone and 11-dehydrocorticosterone in renal cortical and medullary interstitial fluid in conscious, freely moving rats using chronic microdialysis and a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method.
METHODS AND MATERIALS

Animals and tissue harvest. Male Sprague-Dawley rats weighing 250–300 g at the beginning of the experiment were used in the present study. Rats were on a 12:12-h light-dark cycle with lights on from 6 AM to 6 PM. Rats had unlimited access to water. The animal protocols were approved by the Institutional Animal Care and Use Committee of the Medical College of Wisconsin. Tissue harvest and dissection of kidney regions were performed as described previously (23).

Rats were used in four protocols. In protocol 1, 20 rats were uninephrectomized and implanted with femoral arterial catheters, renal medullary interstitial infusion lines, and renal medullary microdialysis probes for studying the effect of carbenoxolone. In protocol 2, 16 rats were implanted with femoral arterial catheters and renal cortical and medullary microdialysis probes (in the left kidney) for the characterization of renal cortical and medullary interstitial glucocorticoids. In protocol 3, 8 rats were instrumented similar to protocol 2 and used to study the effect of dietary high-salt intake. In protocol 4, 10 uninstrumented rats were used for tissue harvest for the analysis of gene expression.

Chronic renal interstitial microdialysis in conscious rats. The renal interstitial microdialysis method we previously used in anesthetized rats (1, 22) was modified for chronic experiments in conscious rats. The inflow and outflow tubes of a linear microdialysis probe (5-mm membrane, 5-kDa molecular mass cutoff, Bioanalytical Systems, West Lafayette, IN) were replaced with PE-10 polyethylene tubes to increase resistance to breakage. Rats were anesthetized briefly with ketamine (40 mg/kg), xylazine (8 mg/kg), and acepromazine (4 mg/kg), and the left kidney was exposed through a flank incision. Probes were placed into the renal cortex and the renal medulla, or the renal medulla only, using a 30-gauge needle. The inflow and outflow tubes were tunneled under the skin and exteriorized at the back of the neck where a spring was attached to protect the tubes. Rats were housed in individual metabolic cages. The inflow tubes were connected to an infusion pump through a double-channel swivel and connected to the renal arterial catheter by 70:30 methanol-water. A four-point calibration curve of 25, 5, 1, and 0.2 ng/ml was made by serial dilutions of the working standard. Corticosterone 2,2,4,6,6,17a,21,21-ds (isotopic enrichment 97%) was purchased from CDN Isotopes (Quebec, Canada) and was used as an internal standard. A 1-µg/ml working internal standard was made in 70:30 methanol-water. A 10-µl internal standard was added to 50 µl of each sample or standard, and an acetonitrile rinse was performed to remove particulate matter and to precipitate proteins. Four milliliters of CHCl₃ were added for organic liquid/liquid extraction. The methylene chloride layer was washed with base, acid, and water before dry-down and resuspension in 50 µl of 70:30 methanol-water containing estriol (to prevent nonspecific binding of the analytes to glassware).

Perkin-Elmer Series 200 pumps and a CTC-Pal Autosampler were used for sample introduction to the mass spectrometer. A reversed-phase analytical column (SUPELCOSIL LC-18; 33 µm, 4.6×150 mm) was used for sample introduction to the mass spectrometer. Samples were introduced to the mass spectrometer in 20-µl volumes. The mobile phase, delivered at 1,000 µl/min, consisted of 45% methanol in 0.1% (vol/vol) ammonium acetate, followed by a 4-min ramp to 85% methanol in 0.1% ammonium acetate.

LC-MS/MS measurements were performed with an API 4000 Q-trap tandem mass spectrometer (Applied Biosystems, Foster City, CA), operating with an electrospray ionization source in the positive mode. The mass spectrometer operating parameters consisted of the source heater probe at 500°C, with an ionspray voltage of 5,000 V, a curtain gas setting of 10, entrance potential of 10 V, and ion spray 1 and 2 voltages at 50 and 40, respectively. Data acquisition and processing were performed using Analyst software (Applied Biosystems). Corticosterone, 11-dehydrocorticosterone, corticosterone-d₄, and cortisol were monitored by ion pairs of 347.3/212.2, 345.2/212.2, 355.2/125.1, and 363.1/121.1, respectively. A multiple reaction monitoring chromatogram is shown in Fig. 1. Signal-to-noise ratios for the 0.2 ng/ml standards of corticosterone, 11-dehydrocorticosterone, and cortisol were 24.2, 18.8, and 17.8, respectively.

Measurement of urinary concentration of Na⁺ and K⁺. Urinary concentrations of Na⁺ and K⁺ were measured using a flame photometer (22).

Real-time PCR. Real-time PCR analysis using the Taqman chemistry (Applied Biosystems) was performed as described previously (17, 24). 18S rRNA was used as an internal normalizer. Oligonucleotide sequences were: rat 11β-HSD1, forward CTTCTCCTGCTGGCTGC- GAAA, reverse AAGAAACCTCAGACGCAAACCT, probe FAM-ACC- CCAACCTGTGCTGCTGGCCTGA-TAMRA; rat 11β-HSD2, forward CTCCTCCTGCTGGCCCAGATTG, reverse CAGCTGTTA CCTCCCTCATGACGCT, probe FAM-CCTACGAGCCGACGC- TTCCAGC-TAMRA.

Western blot. Western blot analysis was performed as described previously (20, 21, 24). A goat antibody for human 11β-HSD1 was obtained from R&D Systems (Minneapolis, MN), catalog number AF3397, and used at a final concentration of 0.5 µg/ml. The antibody detected a distinct band at the expected molecular weight of 11β-HSD1 in samples from both the kidney and the liver, the latter known to express a high level of 11β-HSD1. Two additional bands at higher molecular weights of ~100 KDa were seen, especially in kidney samples. A sheep antibody for rat 11β-HSD2 was obtained from Chemicon International (Millipore, Billerica, MA), catalog number AB1296, and used at 1:2,000 dilution. The antibody detected a single
band at the expected molecular weight of 11β-HSD2. Membranes were stained with Coomassie blue, and the total Coomassie blue intensity in each lane was used for normalization.

**Immunohistochemistry.** Kidneys were cut into two halves along the corticopapillary axis and immediately immersed in 10% neutral formalin. Fixed kidney tissue was processed on SAKURA VIP 30 and embedded with Tisher Tissue Tek 2. Paraffin sections, 3-μm thick, were cut and mounted on Fisher Plus slides. Deparaffinization and hydration were performed using SAKURA DRS2000 Automated Stainer. Endogenous peroxidase and bixinol and nonspecific binding sites were sequentially blocked. Tissue sections were then incubated with the sheep antibody for 11β-HSD2 (Chemicon International, 1:2000 dilution) for 90 min, followed by biotinylated secondary antibody and then streptavidin-peroxidase conjugates. Color was developed using DAB reagents.

**Statistical analysis.** Data were analyzed using ANOVA, Student’s $t$-test, or paired $t$-test when appropriate and are presented as means ± SE. $P < 0.05$ was considered significant.

**RESULTS**

**Effect of renal medullary interstitial infusion of carbenoxolone.** We examined the physiological significance of regional metabolism of glucocorticoids in the kidney. We tested the hypothesis that inhibition of 11β-HSD by carbenoxolone locally in the renal medulla would be sufficient to cause hypertension. Direct infusion of carbenoxolone (6 mg/day) into the renal medullary interstitial space increased mean arterial blood pressure from $110 \pm 2$ mmHg at the baseline to $130 \pm 3$ mmHg at 5 days after the initiation of the infusion ($n = 10$, $P < 0.05$; Fig. 2). Intravenous infusion of carbenoxolone at the same dose did not induce hypertension. Blood pressure was $109 \pm 1$ mmHg at the baseline and $108 \pm 2$ mmHg after 5 days of intravenous infusion ($n = 12$). Intravenous infusion of carbenoxolone at a higher dose, 12 mg/day, increased blood pressure to $127 \pm 3$ mmHg ($n = 9$, $P < 0.05$ vs. baseline) after 2 days. Blood pressure returned to the baseline level of $108 \pm 5$ mmHg after 5 days.

Glucocorticoid levels were analyzed before interstitial carbenoxolone infusion (day 0) and at day 3 and day 7 after the initiation of carbenoxolone treatment. The samples were collected from 2 to 5 PM. The renal medullary infusion of carbenoxolone significantly increased corticosterone concentrations in the renal medullary microdialysate from $1.12 \pm 0.17$ at the baseline to $3.23 \pm 0.59$ ng/ml at 7 days ($n = 8$ to 9, $P < 0.05$ vs. control). The concentrations tended to increase at 3 days but did not reach statistical significance (Fig. 3A). Corticosterone levels did not change significantly in either the plasma or urine. The corticosterone/11-dehydrocorticosterone ratio, an index of 11β-HSD activities, was also significantly increased in the medullary microdialysate by about twofold from the baseline to 7 days (Fig. 3B). The ratio did not change significantly in the plasma or urine.

Urinary Na⁺ concentrations in the carbenoxolone-treated group significantly decreased from $0.20 \pm 0.10$ on day 0 to $0.69 \pm 0.17$ mmol/l on day 3 ($n = 10$, $P < 0.05$) and $0.61 \pm 0.13$ mmol/l on day 7 ($n = 6$, $P < 0.05$). Na⁺ concentrations did not change significantly in the control group. Urinary K⁺ concentrations did not change significantly, although there was a tendency of increase in the carbenoxolone-treated group from day 0 to day 7 ($32.8 \pm 2.3$ vs. $48.1 \pm 10.5$ mmol/l, $n = 6$, NS).

It should be cautioned that dietary intake was not controlled in the present study, and urine volume could not be accurately measured due to the short collection period of 3 h and the use of metabolic cages. Urine volume is not critical for interpreting the glucocorticoid data but is important for interpreting the electrolyte excretion data.

**Interstitial corticosterone and 11-dehydrocorticosterone in the renal cortex and medulla.** The carbenoxolone study suggested that measurement of glucocorticoids in renal interstitial microdialysate had significant value beyond measurements in the plasma and urine. We performed further studies to characterize glucocorticoid levels in both renal cortical and medullary interstitial microdialysate in additional groups of conscious, freely moving, untreated rats.

As shown in Fig. 4A, concentrations of corticosterone (average of morning and afternoon collections) in renal cortical and medullary microdialysate were $0.76 \pm 0.14$ and $1.02 \pm 0.15$ ng/ml, respectively ($n = 15$ to 16, NS). In vitro microdialysis indicated that the relative recovery rate for corticosterone was $15.4 \pm 1.5\%$ ($n = 3$). Assuming the in vivo recovery rate was similar, we estimated that the levels of corticosterone were $69.5 \pm 9.0$ ng/ml in the renal cortex and $91.7 \pm 10.5$ ng/ml in the renal medulla.

**Effect of renal medullary microarterial blood pressure.** Carbenoxolone (6 mg/day) or vehicle was infused directly into the renal medullary interstitium of conscious, uninephrectomized Sprague-Dawley rats, starting after blood pressure recording on day 0. Mean arterial blood pressure (MAP) was monitored daily (2 to 5 PM) using indwelling femoral arterial catheter, $n = 9$ to 10. *$P < 0.05$ vs. day 0.
in renal interstitial fluid would be ~6 ng/ml. Urinary and plasma concentrations of corticosterone were 7.9 ± 1.1 and 66.7 ± 8.1 ng/ml, respectively (n = 15 to 16). Concentrations of 11-dehydrocorticosterone in renal interstitial microdialysate, the plasma, and urine are shown in Fig. 4B. The relative recovery rate for 11-dehydrocorticosterone in vitro was 14.4 ± 2.0% (n = 3). Cortisol was not detectable in the majority of the samples.

The ratio of corticosterone/11-dehydrocorticosterone was 0.83 ± 0.13 and 0.60 ± 0.07 in renal cortical and medullary microdialysate, respectively (n = 13, NS). These ratios were significantly lower than that in urine or the plasma (Fig. 4C).

Regional expression of 11β-HSD1 and 11β-HSD2. We analyzed the regional distribution of 11β-HSD1 and 11β-HSD2 using quantitative Taqman real-time PCR and Western blot with currently available antibodies. Rats used in the expression study were not instrumented or treated. As shown in Fig. 5, both 11β-HSD1 and 11β-HSD2 were readily detectable in the rat renal cortex, renal outer medulla, and renal inner medulla. 11β-HSD1 appeared to be more abundant in the medulla than in the cortex. 11β-HSD2 was most enriched in the outer medulla, followed by the cortex and the inner medulla. Immunohistochemistry analysis showed that 11β-HSD2 was detectable in the collecting duct in the cortex, the outer medulla, and...
the inner medulla, largely consistent with previous findings (18, 34). The 11β-HSD1 antibody we used did not appear suitable for immunohistochemistry analysis (see METHODS AND MATERIALS).

Circadian changes. Circulating levels of glucocorticoid are known to exhibit circadian fluctuations. This was confirmed in our study where plasma levels of corticosterone were ~2.8-fold higher in the afternoon (3:30 PM) than in the morning (10:30 AM; Fig. 6A). The same trend was observed in renal microdialysate but the magnitude of the difference was smaller. Corticosterone levels in renal microdialysate were ~1.6-fold higher in the afternoon than in the morning (Fig. 6A). Urinary corticosterone levels tended to be higher in the afternoon but did not reach statistical significance. The corticosterone/11-dehydrocorticosterone ratio was higher in the afternoon in renal cortical microdialysate, similar to the plasma (Fig. 6B). The ratio did not fluctuate significantly in the medullary microdialysate.

Effect of increased dietary salt intake. We examined the effect of high-salt intake on renal interstitial glucocorticoid levels. Rats were switched from a baseline 0.4% NaCl diet to a 4% NaCl diet for 3 days. The samples were collected from 2

---

Fig. 5. Expression of 11β-HSD1 and 11β-HSD2 in kidney regions. mRNA levels of 11β-HSD1 (A) and 11β-HSD2 (B) were quantified with Taqman real-time PCR and normalized to 18S rRNA. Protein levels of 11β-HSD1 (C) and 11β-HSD2 (D) were measured with Western blot in tissue homogenate and normalized to Coomassie blue staining. Liver was used as a positive control for 11β-HSD1. Data are shown as percentage of average levels in cortex. Representative Western blots of 11β-HSD1 (E, top) and 11β-HSD2 (F, top) and corresponding Coomassie blue staining (bottom) are shown, n = 5 to 10. *P < 0.05 vs. cortex. C, renal cortex; OM, outer medulla; IM, inner medulla.

Fig. 6. Circadian changes of systemic and renal interstitial glucocorticoid levels. Corticosterone levels (A) and corticosterone/11-dehydrocorticosterone ratios (B) were measured in conscious, freely moving rats in mornings (9 AM to noon) and afternoons (2 to 5 PM), n = 13 to 16. *P < 0.05 vs. AM.
to 5 PM. The treatment did not significantly alter renal interstitial levels of corticosterone or corticosterone/11-dehydrocorticosterone ratio. In cortical microdialysate, corticosterone levels were 0.75 ± 0.14 and 0.83 ± 0.18 ng/ml on 0.4 and 4% NaCl, respectively, and the ratio was 1.33 ± 0.42 and 1.31 ± 0.25, respectively (n = 7 to 8, NS). In medullary microdialysate, the corticosterone levels were 1.23 ± 0.39 and 1.15 ± 0.14 ng/ml, respectively, and the ratio was 0.53 ± 0.1 and 0.98 ± 0.28, respectively (n = 7 to 8, NS).

**DISCUSSION**

The present study utilized a new approach combining chronic renal microdialysis, LC-MS/MS analysis, and renal medullary interstitial infusion of an 11β-HSD inhibitor to study local metabolism of glucocorticoids. It demonstrated that corticosterone excess in the renal medulla might be sufficient to cause hypertension and provided the first characterization of renal interstitial glucocorticoids.

Carbenoxolone can inhibit both 11β-HSD1 and 11β-HSD2 (4) and has diverse effects (16, 32). The specific renal medullary administration used in the present study obviates certain confounding effects associated with systemic administration. The findings of the present study indicate that the net effect of renal medullary administration of carbenoxolone is suppression of 11-dehydrogenation of corticosterone. The resulting increase in renal medullary corticosterone could mediate or contribute to the concomitant hypertension. The finding that alterations of corticosterone metabolism in the renal medulla can lead to hypertension is significant since differential expression of 11β-HSDs has been reported in the renal medulla of rat models of hypertension (23). Given the known role of 11β-HSD2 in maintaining mineralocorticoid receptor selectivity (12, 35), hypertension observed in the present study might be due to overstimulation of mineralocorticoid receptors as a result of carbenoxolone infusion. Other mechanisms could also be involved. For example, glucocorticoids have been shown to suppress the expression of endothelial nitric oxide synthase, an effect exacerbated by knockdown of 11β-HSD2 (25).

The large diurnal variation of plasma corticosterone appeared to be partially buffered in the kidney since the variation was smaller in the renal microdialysate. It might reflect the capacity of renal 11β-HSD to maintain a relatively stable level of corticosterone, which might be important given the potential impact of corticosterone on sodium and fluid transport.

There was a tendency for higher corticosterone levels and lower corticosterone/11-dehydrocorticosterone ratios in the medulla compared with the cortex. The differences were not statistically significant. Both 11β-HSD1 and 11β-HSD2 tend to be enriched in the medulla, which could contribute to the complexity of intrarenal regional glucocorticoid metabolism. Medullary glucocorticoid levels did not appear to differ significantly in uninephrectomized rats (the interstitial carbenoxolone groups) compared with rats with two kidneys. It has been reported that renal levels of active glucocorticoids increased at 24 h but not 2 wk after uninephrectomy (9). The design of the present study was more in line with the long-term time point.

The plasma concentrations of corticosterone obtained in the present study are consistent with values previously reported in conscious rats (6, 37). Corticosterone/11-dehydrocorticosterone ratios in rat urine were reported to be about three to four according to ELISA (13) or 0.7 according to a radioimmunoassay (36a), compared with about one to two in the present study.

Urinary steroid measurements have been, and will continue to be, valuable as indexes of renal glucocorticoid metabolism, especially in studies involving humans or large changes in the whole kidney. Tissue levels of glucocorticoid metabolites can also be analyzed using tissue extracts (8). Renal interstitial measurement will be a particularly valuable alternative in experimental animals when the changes are subtle or confined to a kidney region and when temporal changes in conscious animals are of interest. Microdialysis could also be useful in the study of other tissues, such as liver, fat, and brain, where local metabolism of glucocorticoids is important.

Additional factors need to be considered when interpreting the microdialysate data. Interstitial levels of glucocorticoids are likely influenced by both circulating and intracellular levels since glucocorticoids are steroids and can move across the plasma membrane. Circulating corticosterone is largely protein-bound. The reported percentages of rat plasma corticosterone that is unbound vary from ~1% to more than 10% (10, 14). Renal interstitial levels of corticosterone would, therefore, appear to be generally in the same range as plasma levels of the unbound corticosterone. The renal interstitial levels we measured probably reflect the influence of various cell types within a kidney region since the microdialysis membrane is 5 mm long. In collecting duct cells, where 11β-HSD2 is enriched (18, 19), corticosterone levels could be lower.

Quantification of intracellular levels of glucocorticoids with sufficient temporal and spatial resolution will ultimately be needed to truly understand local metabolism of glucocorticoids. Renal microdialysis and LC-MS/MS analysis represent a significant step toward that goal. The approach allowed us to detect changes of corticosterone metabolism in the renal medulla, not reflected in urine, that were associated with hypertension.

**REFERENCES**


F192

RENUAL INTERSTITIAL GLUCOCORTICOIDS


