Effects of dietary salt changes on renal renin-angiotensin system in rats

CATHERINE INGERT,1 MICHELE GRIMA,1,2 CATHERINE COQUARD,1 MARIETTE BARTHELMEBS,1 AND JEAN-LOUIS IMBS1,2

1Institut de Pharmacologie, Faculté de Médecine, Université Louis Pasteur and 2Service d’Hypertension, des Maladies Vasculaires et Pharmacologie Clinique, Hôpitaux Universitaires de Strasbourg, 67085 Strasbourg, France

Received 26 October 2001; accepted in final form 25 June 2002

Ingrer, Catherine, Michelle Grima, Catherine Coquard, Mariette Barthelmebs, and Jean-Louis Imbs. Effects of dietary salt changes on renal renin-angiotensin system in rats. Am J Physiol Renal Physiol 283: F995–F1002, 2002.—Renin (RA) and angiotensin-converting enzyme (ACE) activities and angiotensinogen, ANG I, and ANG II levels were measured in the kidney (cortex and medulla) and plasma of Wistar-Kyoto rats on a low-sodium (LS; 0.025% NaCl; n = 8), normal-sodium (NS; 1% NaCl; n = 7), or high-sodium (HS; 8% NaCl; n = 7) diet for 21 days. RA, ANG I, and ANG II levels increased in a manner inversely related to sodium content of the diet in both plasma and renal tissues. The LS diet resulted in a 16-, 2.8-, and 1.8-fold increase in plasma RA, ANG I, and ANG II levels, respectively, compared with those in HS rats. In the renal cortex and medulla, RA, ANG I, and ANG II levels were also increased by diminution of dietary salt content but, in contrast to plasma, ANG II levels increased much more than RA or ANG I levels [5.4 (cortex)- and 4.7 (medulla)-fold compared with HS rats]. In summary, we demonstrated variations of RA and ANG I, and ANG II levels [5.4 (cortex)- and 4.7 (medulla)-fold compared with HS rats]. In plasma, renin activity (RA) is the factor limiting ANG II synthesis, but no clear evidence exists for such a role of this enzyme in the kidney. The objectives of the present study were 1) to determine the factor limiting ANG II levels in the kidney and 2) to explore a different functionality of RAS in the renal cortex and medulla. For this purpose, we applied a physiological approach to get closer to in vivo conditions. Among physiological factors regulating renin secretion, the level of dietary salt intake is of particular relevance because it is well established that synthesis and secretion of renin are inversely related to salt intake (12). We measured RA, ACE activity, angiotensinogen, ANG I, and ANG II in the renal cortex, and the plasma of rats on a low-sodium (LS), a normal-sodium (NS), or a high-sodium (HS) diet. By measuring the principle components of the RAS simultaneously, we can discuss their respective roles in the key steps of the enzymatic cascade and then determine the factor limiting the RAS in the kidney. By separating the kidney into cortex and medulla, we could investigate a different functionality of RAS in these two tissues, considering the differing localization of angiotensinogen, renin, and ACE as described above.

MATERIALS AND METHODS

Experimental Design

Male Wistar-Kyoto rats were obtained from Iffa-Credo (l’Arbresle, France) and housed in our laboratory 1 wk before the beginning of the treatments. A 12:12-h dark-light cycle

http://www.ajprenal.org 0363-6127/02 $5.00 Copyright © 2002 the American Physiological Society
was used (lights on from 6 AM to 6 PM). Animals had free access to tap water and consumed a diet of rat chow (UAR, Epinay sur Orge, France). For the experiments with an altered dietary salt intake, the animals were fed a low-salt (LS group, 0.025% NaCl; n = 8), a normal-salt (NS group, 1% NaCl; n = 7), or a high-salt (HS group, 8% NaCl; n = 7) diet for 21 days. The different salt diets (UAR 212, UAR 210, and UAR 210+, respectively) and distilled drinking water were available ad libitum. All animals were 10 wk old on the day of sampling and were deprived of food but not of water the night before plasma and tissue sampling. Because anesthesia induces a strong stimulation of RAS, rats were killed by decapitation.

**Plasma and Kidney Sampling**

After decapitation, ~5 ml of trunk blood were rapidly collected into ice-cold tubes containing 0.25 ml of inhibitor cocktail (2.5 mM phenanthroline, 3 mM EDTA, 0.1 mM pepstatin, 3 μM rat renin inhibitor Ac-His-Pro-Phe-Val-Sta-Leu-Phe-NH₂ (Néosystem), 0.11 mM neomycin sulfate; final concentrations) for measurement of ANG I and ANG II; 1 ml was used for plasma renin activity (PRA) and plasma angiotensinogen (EDTA tube), and 1 ml was used for ACE activity (lithium heparin tube). The blood was immediately centrifuged at 4°C for 10 min at 2,000 g. Plasma samples were frozen in liquid nitrogen and stored at −80°C until assay.

After decapitation, kidneys were immediately removed and bisected. Each half of the right kidney was rapidly dissected on an ice-cold plate into cortex and medulla. Micrlosic care was taken to cut fine strips of the outer cortex and to restrict the medulla to its internal section to limit the presence of juxtapeludar glomerules. One-half of each tissue sample was used for measurement of ANG I and ANG II; these were weighed and subsequently homogenized in ice-cold methanol (9). The homogenates were centrifuged at 4°C for 10 min at 2,000 g, and the supernatants were evaporated to dryness and stored at −80°C. The other half of each tissue sample was used for determination of renal RA and was homogenized in a phosphate buffer with 144 mM PMSF-10 mM phenylmercuric acetate (in ethanol) and centrifuged at 4°C for 20 min at 5,000 g (6). The supernatants were stored at −20°C until assay.

Each half of the left kidney was also dissected into cortex and medulla and weighed. One-half of each tissue sample was used for determination of renal angiotensinogen and homogenized in a phosphate buffer with 1.5 M ammonium sulfate, 1.5 mM pepstatin (in ethanol), and 144 mM PMSF-10 mM phenylmercuric acetate (in ethanol); the homogenate was centrifuged at 4°C for 20 min at 5,000 g, and the supernatant was decanted. To precipitate angiotensinogen, the ammonium sulfate concentration was adjusted to 2.5 M by addition of 4 M ammonium sulfate and the supernatant was centrifuged as before. The ammonium sulfate precipitate was resuspended in water and stored at −20°C until assay (6). The other half of each tissue sample was frozen in liquid nitrogen; after thawing of the cortex and the medulla, Triton X-100 (0.3%) was added and the tissues were homogenized and centrifuged at 4°C for 20 min at 11,500 g after sonication. The supernatants were diluted in Triton X-100 for determination of ACE activity and stored at −20°C until assay.

**Measurement of RAS Components**

**Plasma and renal angiotensinogen.** Plasma angiotensinogen was determined by incubating diluted plasma with a phosphate buffer, a pool of exogenous rat renin, and 144 mM PMSF (in ethanol) (6). After incubation at 37°C for 60 min, ANG I generation was quantified by RIA as described below. Cortical and medullar angiotensinogen samples were incubated with a phosphate buffer, a pool of exogenous rat renin, and 144 mM PMSF-10 mM phenylmercuric acetate (in ethanol). After incubation at 37°C for 120 min, 0.5 ml of 1% trifluoroacetic acid was added; each incubation was extracted with a Sep-Pak C₁₈ cartridge (Oasis Waters, Milford, MA), the eluate was evaporated to dryness, and ANG I was determined by RIA (6). Plasma and renal angiotensinogen are expressed as nanograms of ANG I generated per milliliter of plasma and nanograms of ANG I generated per gram of tissue weight, respectively.

**Plasma and renal tissue renin activities.** PRA was measured by determining the level of ANG I generated during a 30-min incubation of plasma at 37°C in the presence of 5 mM 8-hydroxyquinoline. ANG I was measured by RIA. In the cortex and the medulla, RA was measured after determination of ANG I generated during a 30-min incubation of diluted supernatants in the presence of plasma enriched in angiotensinogen obtained from rats 48 h after uninephrectomy (6). ANG I was measured by RIA. PRA and renin RA are expressed as nanograms of ANG I generated per milliliter of plasma per hour and micrograms of ANG I generated per gram of tissue weight per hour, respectively.

**Plasma and renal ACE activities.** ACE activity was determined in vitro with an enzymatic method. In vitro plasma and renal ACE activities were determined as described by Unger et al. (35) in the presence of an artificial substrate (10 mM N-carbobenzoxy-Phe-His-Leu, 67 mM phosphate buffer, pH 8.0, 300 mM NaCl, 10 μM ZnSO₄). The dipeptide (His-Leu) produced by the reaction was measured spectrofluorimetricaly after coupling with o-phthaldialdehyde. To ensure linearity of the ACE activity measurement, the protein concentration in the assay was maintained at <2 mg/ml (cortex or medulla) or <20 mg/ml (plasma) (36). ACE activity is expressed as nanomoles of His-Leu formation per minute per milligram of protein.

**Plasma and renal angiotensins.** Plasma angiotensins were extracted by reversible adsorption to phenylsil-silica (Bondelut-PH; Analytichem, Harbor City, CA), and ANG II was additionally separated from other peptides by isocratic reversed-phase HPLC (Nucleosyl 100–5C; Macherey-Nagel, Oensingen, Switzerland) according to the method of Nussberger et al. (26). ANG I and ANG II levels were quantified by RIA with rabbit anti-ANG I and anti-ANG II sera. Antibodies to ANG I and ANG II were raised in our laboratory in rabbits immunized against the peptide coupled to BSA by carbodiimide condensation (21). Renal angiotensins were determined with slight modifications of the method described by Fox et al. (9). Briefly, the dried residues were dissolved in a phosphate buffer containing 267 mg/l BSA. Renal angiotensins were extracted by reversible adsorption to phenylsil-silica (Bondelut-PH) and quantified by RIA with rabbit anti-ANG I and anti-ANG II sera. Results are reported in femtomoles per milliliter of plasma or in femtomoles per gram of tissue weight.

We verified that blood contamination did not contribute to our measurements of renal RAS components. For this purpose, we compared the renal cortex and medulla RAS (RA, ACE activity, ANG I and ANG II levels) of rats whose kidneys were rinsed or not with physiological serum (data not shown). Renal cortex and medulla were dissected in the same conditions as described in Plasma and Kidney Sampling. The results showed that there was no difference between cortex and medulla of kidneys rinsed or not for all the measured components of the RAS. Thus blood contamination does not seem to play a significant part in renal RAS.
**Statistical Analysis**

Data are means ± SE. For each parameter, control and treated groups were compared with a one-way ANOVA followed by Tukey’s multiple-comparison test (SIGMA STAT; SPSS, Chicago, IL). Differences were considered statistically significant at $P < 0.05$ levels.

**RESULTS**

**Effects of Dietary Salt Changes on Different Components of RAS in Plasma**

The LS diet led to a decrease in plasma angiotensinogen levels compared with control (NS) diet (843.31 ± 41.48 vs. 990.50 ± 35.64 ng ANG I/ml; $P < 0.05$) and to a marked increase in PRA (97.87 ± 6.22 vs. 21.82 ± 1.21 ng ANG I·ml⁻¹·h⁻¹; $P < 0.001$), ANG I (177.54 ± 30.10 vs. 84.81 ± 5.82 fmol/ml; $P < 0.01$) and ANG II (210.99 ± 29.32 vs. 135.41 ± 22.28 fmol/ml; $P < 0.05$) levels (Fig. 1). The LS diet did not modify plasma ACE activity or the ANG II-to-ANG I ratio. The HS diet did not significantly change plasma angiotensinogen levels, ANG I and ANG II levels, or the ANG II-to-ANG I ratio compared with the NS diet (Fig. 1). The HS diet decreased PRA (6.16 ± 1.28 vs. 21.82 ± 1.21 ng ANG I·ml⁻¹·h⁻¹; $P < 0.001$) and increased ACE activity (5.71 ± 0.14 vs. 4.87 ± 0.25 nmol His-Leu·min⁻¹·mg protein⁻¹; $P < 0.01$) compared with the NS diet.

**Effects of Dietary Salt Changes on Different Components of RAS in Renal Cortex**

The LS diet led to a decrease in cortical angiotensinogen levels (101.82 ± 7.74 vs. 156.70 ± 14.68 ng ANG I/g tissue; $P < 0.05$) and increased cortical RA (363.25 ± 28.08 vs. 184.47 ± 6.81 µg ANG I·g tissue⁻¹·h⁻¹; $P < 0.001$), ANG I levels (503.13 ± 29.56 vs. 317.53 ± 16.99 fmol/g tissue; $P < 0.001$), ANG II levels (815.76 ± 31.85 vs. 306.58 ± 25.09 fmol/g tissue; $P < 0.001$), and the ANG II-to-ANG I ratio (1.66 ± 0.13 vs. 0.96 ± 0.05; $P < 0.001$) compared with the NS diet (Fig. 2). The LS diet did not modify cortical ACE activity.

The HS diet did not change cortical angiotensinogen levels, RA, the ANG II-to-ANG I ratio, or ACE activity compared with the NS diet. The HS diet decreased ANG I (196.92 ± 22.26 vs. 317.53 ± 16.99 fmol/g tissue; $P < 0.01$) and ANG II (151.24 ± 21.28 vs. 306.58 ± 25.08 fmol/g tissue; $P < 0.001$) levels compared with the NS diet (Fig. 2).

**Effects of Dietary Salt Changes on Different Components of RAS in Renal Medulla**

Salt restriction led to a decrease in medullar angiotensinogen levels (87.87 ± 6.26 vs. 134.41 ± 6.78 ng ANG I/g tissue; $P < 0.001$) and increased medullary RA (151.84 ± 6.62 vs. 58.02 ± 2.71 µg ANG I·g tissue⁻¹·h⁻¹; $P < 0.001$), ANG I levels (437.78 ± 12.75 vs. 325.96 ± 22.32 fmol/g tissue; $P < 0.001$), and ANG II levels (860.15 ± 57.93 vs. 345.86 ± 23.83 fmol/g tissue; $P < 0.001$), and the ANG II-to-ANG I ratio (1.97 ± 0.15 vs. 1.07 ± 0.09; $P < 0.001$) compared with the NS diet (Fig. 3). The LS diet did not modify ACE activity in the medulla.

The HS diet did not change medullary angiotensinogen levels, the ANG II-to-ANG I ratio, or ACE activity compared with the NS diet. The HS diet decreased RA (27.30 ± 2.63 vs. 58.02 ± 2.71 µg ANG I·g tissue⁻¹·h⁻¹; $P < 0.001$), ANG I levels (224.05 ± 6.87 vs. 325.96 ± 22.32 fmol/g tissue; $P < 0.001$), and ANG II levels (182.66 ± 20.80 vs. 345.86 ± 23.83 fmol/g tissue; $P < 0.05$) compared with the NS diet (Fig. 3).

The differences observed in LS rats compared with NS rats were similar but amplified compared with HS rats for the complete RAS in plasma, renal cortex, and renal medulla.

**DISCUSSION**

In the present study, we analyzed the variation of the different components of the RAS in kidney and plasma of rats subjected to RA changes induced by high- or low-salt intake. Our findings confirm that ANG II levels are high in kidney compared with plasma in relation to changes in dietary salt intake.

As expected, PRA was stimulated by salt restriction and decreased by salt excess. The magnitude of changes of PRA observed after salt restriction is in agreement with prior reports (14, 22, 28). This increase in PRA could result from a decrease in tubular sodium in the macula densa, resulting in a release of renin from juxtaglomerular cells (22). With an 8% NaCl diet, we expected a suppression of PRA as shown by Nishimura et al. (25), but our results did not confirm this. Salt restriction also leads to a decrease in plasma angiotensinogen levels, which can be explained by marked elevation of circulating renin and/or by modifications of angiotensinogen synthesis and secretion from the liver (22). Several studies showed that a low salt intake did not influence the expression of hepatic angiotensinogen mRNA (18, 27). We suggest that the decrease in angiotensinogen levels induced by salt restriction observed in the plasma could be due to a higher consumption of angiotensinogen by the elevated plasma renin. Some authors found much higher ANG I levels than measured in the present study (2, 9). This was probably due to a failure to completely inhibit renin during plasma sample processing, which may result in artifactually high levels of ANG I. In our study, we used pepstatin and a renin inhibitor as used by Campbell et al. (4–6) to limit this phenomenon. Three weeks of salt restriction were associated with an elevation of plasma ANG I and ANG II levels. This elevation followed the marked increase in PRA. Plasma ACE activity was slightly modified by dietary salt changes but did not seem to play a limiting role in ANG II synthesis because the plasma ANG II-to-ANG I ratio was not affected. Therefore, we confirm that plasma ACE activity is not the limiting factor for circulating ANG II levels. Together, our findings confirm that renin is the limiting factor of ANG I and ANG II synthesis in the plasma.
RA in the cortex increased in LS and decreased in HS animals as in plasma; these results confirm other studies (9, 15). Sodium deprivation does not induce parallel increases in plasma renin levels and renin biosynthesis as shown by a previous report (24). In fact, an increase in renal RA is merely due to higher synthesis and storage whereas an increase in PRA reflects a release of renin. The high levels of ANG I in the renal cortex compared with those in the plasma suggest that renal tissue ANG I is not due to a simple contamination from plasma but may be due to a local synthesis. Nevertheless, despite high cortical RA induced by salt restriction, ANG I levels were relatively weak. It should be noted that the RA we measured in the kidney is the combination of renin stocked in vesicles and free renin that is only available to act on angiotensinogen. We also suggest that angiotensinogen could be a limiting factor for ANG I synthesis through its low quantity available in the kidney; indeed, cortical angiotensinogen levels were sixfold lower than those measured in the plasma. On the other hand, ANG I synthesis requires the colocalization of angiotensinogen and renin; renin is mainly located in the juxtaglomerular...
apparatus into vesicles despite the findings of Henrich et al. (13) and Moe et al. (23) that cultured proximal tubule cells produce renin in small quantities and contain renin mRNA. Most intrarenal angiotensinogen mRNA and protein have been located in proximal tubule cells (7, 16, 18). Thus angiotensinogen and renin are colocalized in limited renal areas such as lymph or proximal tubules. Therefore, independently from renin and angiotensinogen quantities, their colocalization could limit renal ANG I synthesis. During dietary sodium changes, a similar pattern was observed for angiotensinogen levels in the cortex compared with the plasma. This contrasts with data showing that in the liver sodium deprivation stimulated and sodium excess decreased renal angiotensinogen mRNA expression (18, 20, 32). Therefore, we suggest that the decrease in cortical angiotensinogen induced by sodium restriction may reflect its extensive consumption by high renin levels. Concerning ANG II levels in control rats, we observed threefold higher levels in the cortex than in the plasma, which probably excludes plasma contamination. Cortical ANG II levels increased in the same way as ANG I, which could suggest an intrarenal
synthesis. However, during sodium restriction, ANG II levels increased much more than ANG I levels: 2.7-fold for ANG II vs. 1.6-fold for ANG I compared with the control diet. This led to an increase in the ANG II-to-ANG I ratio that could not be explained by an increase in ACE activity, which was not modified. Of course, we cannot exclude an ACE-independent way to produce ANG II; nevertheless, studies using ANG II perfusions showed that a significant part of intrarenal ANG II resulted from the uptake of circulating and/or intrarenal produced ANG II via the internalization of AT1 receptors (37, 38, 39). Therefore, we suggest that the strong increase in ANG II levels we observed in the cortex during low-salt intake could be due, in part, to the uptake phenomenon that would occur here under more physiological conditions than during ANG II infusion.

In the medulla, we observed different amounts of RA and ACE activity compared with the renal cortex. RA of control rats was threefold lower than that observed in the cortex. Because the presence of renin is highly
localized in the juxtaglomerular apparatus located in the cortex, one can wonder about the origin of renin in the medulla. Renin can reach the medulla via microcirculation or lymphatics (8). Renin has also been described in pinocytotic vacuoles in the proximal straight tubule and the connecting tubules (11). Recent data demonstrate specific binding sites for renin in the medulla, which have the capacity to bind renin threefold lower than in the cortex (30). Finally, renin could also result from a local synthesis in the medulla; in fact, studies using Northern blot analysis demonstrated the presence of renin mRNA in the medulla but in low quantity (18); these studies also showed that RA was influenced by dietary salt changes. To our knowledge, no angiotensinogen levels have been reported in the rat kidney medulla; they were equivalent to those found in the cortex. These results were rather unexpected because angiotensinogen mRNA is mainly located in proximal tubules (19) whereas the presence of small amounts of angiotensinogen mRNA were described in glomerules and vasa recta (34). ANG II contents were equivalent in the cortex and medulla despite a fivefold higher ACE activity and threefold lower RA in the medulla, suggesting that ACE and RA are not the steps limiting ANG II levels. We found similar responses for all parameters of the RAS in the renal medulla compared with those found in the cortex during dietary salt changes. Thus, despite a different localization of angiotensinogen, renin and ACE in the renal cortex and medulla, ANG I and ANG II levels in these two tissues are equivalent and respond in a similar trend to altered sodium intake; these results suggest a similar functionality in these two anatomic parts of the kidney.

In summary, our results confirm that neither RA nor ACE activity is a factor limiting ANG II levels in the kidney. We propose that the factor limiting renal ANG II levels could be angiotensinogen through its low quantity available in the kidney or through its colocalization with renin restricted to limited renal areas. Nevertheless, we could not provide clear evidence for the existence of a compartmentalization of the RAS in the kidney: indeed, despite RA and ACE activity differences between renal cortex and medulla, ANG I and ANG II levels are equivalent in both tissues and respond in a similar trend to dietary salt intake. These results argue against a compartmentalization of the RAS in the renal cortex and medulla. Surprisingly, during sodium restriction, intrarenal ANG II levels increased more than ANG I levels. We suggest that renal ANG II levels result not solely from a local synthesis of ANG I but also from an uptake of circulating and/or intrarenally produced ANG II via AT1 receptors; this should be confirmed by complementary experiences with an AT1 receptor antagonist.

REFERENCES


renin concentration, renal renin content, and rat renin messen-


