Age-Related Changes in Renal Cyclic Nucleotides and Eicosanoids in Response to Sodium Intake

Lesley J. Millatt, Helmy M. Siragy

Abstract—The signaling molecules cGMP, cAMP, prostaglandin E\(_2\) (PGE\(_2\)), and prostaglandin F\(_{2\alpha}\) (PGF\(_{2\alpha}\)) play important roles in mediating the response of the kidney to changes in dietary sodium intake. We used a renal microdialysis technique in conscious rats to address the hypothesis that the renal ability to produce these mediators in response to dietary sodium intake is altered during maturation. Young (4-week-old) or adult (6-month-old) rats were studied after the consumption for 5 days of diets containing low (0.04% NaCl), normal (0.28% NaCl), or high (4.0% NaCl) levels of sodium. Plasma renin activity was significantly increased by low-sodium diet and significantly decreased by high-sodium diet, with no significant difference between the responses of the 2 age groups. Renal interstitial fluid (RIF) levels of cGMP, cAMP, PGE\(_2\), and PGF\(_{2\alpha}\) on normal-sodium diet were similar in the 2 age groups. Low-sodium diet caused a significant increase in RIF levels of all 4 mediators, with no significant differences between the responses of the 2 age groups. High-sodium diet also caused a significant increase in RIF levels of all 4 mediators. However, RIF production of cGMP, cAMP, and PGE\(_2\) was significantly greater, and RIF PGF\(_{2\alpha}\) production was significantly lower, in young rats compared with adult rats. These data demonstrate that the kidneys of young and adult rats respond to dietary sodium restriction in a similar manner but that there are age-related changes in the renal response to sodium loading. (Hypertension. 2000;35:643-647.)

Key Words: age ■ cyclic AMP ■ cyclic GMP ■ kidney ■ prostaglandins ■ sodium ■ rats

The kidney plays a pivotal role in maintaining a constant total body sodium and extracellular fluid volume in the face of changing levels of sodium intake. Increasing age is associated with a number of changes in renal function, including a reduced ability to excrete salt and water loads.\(^1\) The frequency and severity of hypertension increases with age, and even in the absence of hypertension, an increase in the salt sensitivity of blood pressure over time has been observed.\(^3\) Several lines of evidence suggest not only that renal function is altered before the development of hypertension but also that renal dysfunction is a requirement for hypertension to develop.\(^3\) Hence, the decline in renal function with increasing age may explain the increased incidence of hypertension with age. Given that hypertension is more common in adults than in children, we hypothesized that the ability of the kidney to respond to changes in dietary sodium intake may be altered during maturation. Previous studies of the effect of age on renal function have often examined the urinary content of mediators, such as nitric oxide (NO), cyclic nucleotides, and eicosanoids, as an estimate of renal levels of synthesis. However, urinary excretion levels may not directly correlate with renal production because of extrarenal sites of production and alterations in tubular reabsorption.\(^5\) Therefore, in the present study, we used a renal interstitial fluid (RIF) microdialysis technique to directly determine the intrarenal levels of several mediators that play important homeostatic roles in the kidney. We examined the levels of cortical RIF cGMP, cAMP, prostaglandin E\(_2\) (PGE\(_2\)), and prostaglandin F\(_{2\alpha}\) (PGF\(_{2\alpha}\)) produced by young (4-week-old) and adult (6-month-old) Sprague-Dawley rats in response to increased or decreased dietary sodium intake.

Methods
Renal Microdialysis Technique
For the determination of RIF cGMP, cAMP, PGE\(_2\), and PGF\(_{2\alpha}\), we constructed renal microdialysis probes with a molecular mass cutoff of 10 kDa, as previously described.\(^4\) The best in vitro recovery rates for all compounds analyzed were obtained with a perfusion rate of 3 \(\mu\)L/min and were 70% for cGMP,\(^7\) 68% for cAMP, 63% for PGE\(_2\),\(^7\) and 60% for PGF\(_{2\alpha}\).\(^9\)

Animal Preparation
Experiments were conducted with 4-week-old (young group) and 6-month-old (adult group) female Sprague-Dawley rats (n=10 per age group) purchased from Harlan Teklad (Madison, Wis). Both groups were housed under controlled conditions (temperature 21±1°C, humidity 60±10%, and lighting from 8:00 AM to 8:00 PM). All procedures were conducted with the approval of the Animal Research Committee of the University of Virginia.

For the implantation of microdialysis probes, rats were anesthetized with ketamine (80 mg/kg IM) and xylazine (8 mg/kg IM), and

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the right and left kidneys were exposed via a midline abdominal incision. Microdialysis probes were placed in the cortex of both kidneys, as previously described.6–9 To obtain vascular access, a heparinized polyethylene tube was inserted into the right jugular vein. This tube was flushed daily with 10% heparin in 5% dextrose in water and was capped with a small piece of copper wire. After surgery, rats were allowed 7 days for recovery before experiments began.

Experiments were started at the same time (8:00 AM) each day to avoid any diurnal variation of the measured parameters. For RIF collection, the inflow tube of each dialysis probe was connected to a syringe filled with lactated Ringer’s solution and perfused at 3 μL/min. After a 30-minute equilibration period, the effluent from the outflow tube was collected for four 30-minute sampling periods into plastic tubes. Samples were stored at −80°C until the time of assay.

**Effect of Low, Normal, or High Dietary Sodium Intake**

During the 7-day recovery period after surgery, rats consumed a normal-sodium diet (0.28% NaCl). At the end of the recovery period, systolic blood pressure (SBP) was measured by tail-cuff plethysmography (Rat Tail Monometer-Tachometer system, Natsume model KN-210, Peninsula Laboratories), and RIF samples were collected. After the last RIF collection period, a 0.5 mL blood sample for measurement of plasma renin activity (PRA) was withdrawn from the jugular vein catheter of each rat into an EDTA-containing tube. Plasma was separated by centrifugation and stored at −80°C. Rats then consumed either a low-sodium diet (0.04% NaCl) or a high-sodium diet (4.0% NaCl), in random order. Each diet was consumed for 5 days, after which SBP was measured again, and further RIF and plasma samples were collected from each rat. At the end of the study, each kidney was examined to verify the location of the dialysis fibers.

**Analytical Methods**

RIF cGMP, cAMP, PGE$_2$, and PGF$_{2a}$ levels in dialysate samples were measured by use of enzyme immunoassay kits (Cayman Chemical Co). The sensitivities and specificities of the immunoassays were, respectively, 0.11 pmol/mL and 100% for cAMP, 11.4 pg/mL and 100% for PGE$_2$, and 14.2 pg/mL and 100% for PGF$_{2a}$. Cross-reactivity with other cyclic nucleotides was <0.01% for both the cGMP and the cAMP assay. Cross-reactivities of the PGE$_2$ and PGF$_{2a}$ assays with other eicosanoids were <0.01% and 5%, respectively, with PGD$_2$ and <2% with other eicosanoids for the PGF$_{2a}$ assay. PRA was determined by radioimmunoassay,^{10} and activity was expressed as nanograms angiotensin (Ang) I generated per milliliter plasma per hour.

**Statistical Analysis**

Comparisons between different diets were examined by ANOVA, including repeated-measures analysis, with the use of the general linear models procedure of the Statistical Analysis System. Multiple comparisons of individual pairs of effect means were conducted by the use of least squares pooled variance. Data are expressed as mean±SE. A value of $P<0.05$ was considered statistically significant.

**Results**

**Blood Pressure Response to Changes in Dietary Sodium Intake**

During normal-sodium intake, there was no significant difference in SBP between young and adult rats (106±1.5 and 107±1.8 mm Hg, respectively). The consumption of a low- or high-sodium diet for 5 days did not significantly alter the SBP of either young or adult rats (low-sodium diet 107±1.5 and 107±1.7 mm Hg, respectively; high-sodium diet 107±1.5 and 107±1.5 mm Hg, respectively).

**PRA in Response to Changes in Dietary Sodium Intake**

During normal-sodium intake, there was no significant difference in PRA between young and adult rats (1.6±0.5 and 1.5±0.6 ng/mL per hour, respectively) (Figure 1). The consumption of a low-sodium diet for 5 days resulted in a significant ($P<0.0001$) increase in PRA in both young and adult rats (7.8- and 8.7-fold increase, respectively). In contrast, the consumption of a high-sodium diet for 5 days resulted in a significant ($P<0.0001$) decrease in PRA in both young and adult rats (5.3- and 7.5-fold decrease, respectively). There were no significant differences between the PRA values of young and adult rats on either of the altered sodium diets.

**RIF Cyclic Nucleotide Levels in Response to Changes in Dietary Sodium Intake**

During normal-sodium intake, there was no significant difference in either RIF cGMP or RIF cAMP production between young and adult rats (cGMP 1.3±0.4 and 0.9±0.5 pmol/min, respectively; cAMP 1.3±0.5 and 1.0±0.5 pmol/min, respectively) (Figure 2). The consumption of a low-sodium diet for 5 days resulted in a significant ($P<0.0001$) increase in RIF cGMP and cAMP production in both young and adult rats (cGMP 1.9- and 2.1-fold increase, respectively; cAMP 2.0- and 2.2-fold increase, respectively). There were no significant differences in RIF cGMP and cAMP concentrations between young and adult rats after 5 days of low-sodium diet. The consumption of a high-sodium diet for 5 days also caused a significant ($P<0.0001$) increase in RIF cGMP and cAMP production in both young and adult rats (cGMP 3.0- and 2.2-fold increase, respectively; cAMP 5.1- and 3.9-fold increase, respectively). In contrast to the effects of a low-sodium diet, the amounts of RIF cGMP and cAMP produced by young rats after 5 days of high-sodium diet were significantly higher than the amounts produced by adult rats on the same diet ($P<0.001$).
respectively; PGF$_2$α 0.7±0.4 and 1.8±0.5 pg/min, respectively) (Figure 3). The consumption of a low-sodium diet for 5 days resulted in a significant ($P<0.0001$) increase in RIF PGE$_2$ and PGF$_2$α production in both young and adult rats (PGE$_2$ 4.6- and 5.0-fold increase, respectively; PGF$_2$α 18.0- and 6.2-fold increase, respectively). There were no significant differences in RIF PGE$_2$ and PGF$_2$α concentrations between young and adult rats after 5 days of low-sodium diet. The consumption of a high-sodium diet for 5 days also caused a significant ($P<0.0001$) increase in RIF PGE$_2$ and PGF$_2$α production in both young and adult rats (PGE$_2$ 7.0- and 5.4-fold increase, respectively; PGF$_2$α 3.1- and 2.6-fold increase, respectively). There were no significant differences in RIF PGE$_2$ and PGF$_2$α concentrations between young and adult rats after 5 days of low-sodium diet. The consumption of a high-sodium diet for 5 days also caused a significant ($P<0.0001$) increase in RIF PGE$_2$ and PGF$_2$α production in both young and adult rats (PGE$_2$ 4.6- and 5.0-fold increase, respectively; PGF$_2$α 18.0- and 6.2-fold increase, respectively). There were no significant differences in RIF PGE$_2$ and PGF$_2$α concentrations between young and adult rats after 5 days of low-sodium diet. The consumption of a high-sodium diet for 5 days also caused a significant ($P<0.0001$) increase in RIF PGE$_2$ and PGF$_2$α production in both young and adult rats (PGE$_2$ 7.0- and 5.4-fold increase, respectively; PGF$_2$α 3.1- and 2.6-fold increase, respectively). Similar to the changes in cyclic nucleotide production, the amounts of RIF PGE$_2$ produced by young rats after 5 days of high-sodium diet were significantly higher than the amounts produced by adult rats on the same diet ($P<0.001$). In contrast, the amounts of RIF PGF$_2$α produced by young rats after 5 days of high-sodium diet were significantly lower than the amounts produced by adult rats on the same diet ($P<0.001$).

**Discussion**

The major findings of the present study are that young and adult rats respond to dietary sodium restriction in a similar manner, with significant increases in the renal production of the cyclic nucleotides cGMP and cAMP and the eicosanoids PGE$_2$ and PGF$_2$α. In contrast, a high dietary sodium intake resulted in significantly increased renal production of cGMP, cAMP, and PGE$_2$ and significantly decreased renal production of PGF$_2$α in young rats compared with adult rats.

After 5 days of dietary sodium restriction, we observed a 2-fold increase in RIF cyclic nucleotide levels in both young and adult rats. We have previously shown that sodium depletion results in increased cortical production of cGMP.$^7$ This increase is mediated by elevated Ang II produced because of the activation of the renin-angiotensin system during sodium depletion. Ang II acts via the Ang II type 2 (AT$_2$) receptor to stimulate renal production of bradykinin, which activates endothelial NO synthase and leads to increased production of NO and its downstream mediator cGMP.$^8,11$ These responses enable increased renal production of vasodilator cGMP, which counterbalances the greatly increased renal Ang II levels produced during sodium restriction. Dietary sodium restriction is also typically associated with increased pituitary production of the antidiuretic hormone vasopressin. Stimulation of the vasopressin V$_2$ receptor on renal epithelial cells leads to activation of adenylyl cyclase$^{12}$ and may be responsible for the increased RIF cAMP levels observed. In addition, a low-sodium diet was associated with increased RIF PGE$_2$ levels. Within the kidney, PGE$_2$ interacts with 4 distinct G protein–coupled receptors known as EP$_1$ to EP$_4$. The EP$_2$ receptor, which stimulates adenylyl cyclase, has been localized to the glomeruli.$^{13}$ Therefore, it seems likely that the elevated RIF cAMP levels
observed during sodium restriction in the present study occurred in response to increased production of both vasopressin and PGE₂.

We found that 5 days of dietary sodium restriction resulted in a significant increase in RIF eicosanoid levels in both young and adult rats. We have previously shown that sodium depletion causes an increase in RIF PGE₂ levels; this increase was mediated via Ang II stimulation of the Ang II type 1 (AT₁) receptor. Furthermore, sodium depletion is associated with an increase in renal conversion of PGE₂ to PGF₂α, an effect mediated via the AT₂ receptor. PGE₂ plays a major role in the inhibition of tubular sodium reabsorption, thus leading to increased sodium excretion. The increased renal conversion of PGE₂ to PGF₂α during sodium depletion is thus thought to be a protective mechanism to prevent sodium wasting.

Taken together, these results indicate that the adult kidney is able to respond as effectively as the young kidney to dietary sodium restriction. Renal cGMP, cAMP, and PGE₂ increase as a protective measure to counterbalance the increased Ang II levels. To prevent overcompensation by excessive PGE₂ activity, there is also an increase in the conversion of PGE₂ to the vasoconstrictor PGF₂α. All of these homeostatic mechanisms appear to be active in the adult kidney and in the young kidney to a similar degree.

In contrast to the results obtained during dietary sodium restriction, we found that young rats produced significantly higher levels of RIF cyclic nucleotides in response to a high-sodium diet than did adult rats. A well-established mediator of increased renal cGMP production during high-sodium intake is atrial natriuretic peptide (ANP), which acts via the type A guanylyl cyclase–linked natriuretic peptide receptor to stimulate renal cGMP formation. It seems unlikely, however, that a reduced production of ANP could account for the decreased cGMP response to sodium loading in adult rats, in view of the fact that previous studies have reported no difference in either basal or stimulated plasma ANP levels between young and mature rats. Similarly, a reduced renal cGMP response to ANP stimulation is not a likely explanation, because the acute infusion of ANP conversely causes a greater increase in urinary cGMP excretion in 10-week-old than in 4-week-old rats. Another potential explanation for the increased RIF cGMP levels observed in adult rats, in view of the fact that this activity is downregulated in young rats, is the finding that the kidneys of adult rats show a reduced tubular uptake of circulating dopa, resulting in lower renal dopamine levels. Renal production of adenosine is also markedly increased in both the cortex and medulla during high-sodium intake. Within the kidney, adenosine both inhibits adenylyl cyclase via the antinatriuretic A₁ receptor and stimulates adenylyl cyclase via the natriuretic A₂A and A₂B receptors. In view of the recent finding that salt loading downregulates renal expression of the A₁ receptor, without altering expression of the A₂A and A₂B receptors, it seems likely that adenosine contributes to the increased RIF cAMP levels observed in response to a high-sodium diet. Therefore, it will be of interest in the future to determine whether age affects this renal adenosine receptor response to increased sodium load.

In the present study, a high-sodium diet and a low-sodium diet were found to increase cortical RIF PGE₂ levels to a similar degree. It is likely that the increased production of the vasodilator eicosanoid during high sodium intake enhances the ability of the kidney to excrete the sodium load. In addition to the direct conversion of arachidonic acid by the cyclooxygenase enzymes, prostaglandin release may be induced by cytochrome P450–dependent metabolites of arachidonic acid. Thus, it is possible that the increased RIF PGE₂ levels observed in the present study were due to salt-induced upregulation of renal cytochrome P450. In this regard, it is of interest that renal cytochrome P450–dependent metabolism of arachidonic acid has been found to decline during maturation, providing a potential explanation for the present finding that young rats produce significantly greater amounts of PGE₂ in response to high-sodium diet than do adult rats. Moreover, the increased RIF PGE₂ produced by young rats during high-sodium intake is likely to act via the EP₂ receptor to contribute to the increased RIF cAMP levels observed. Indeed, the importance of the EP₂ receptor in the renal response to sodium loading was recently demonstrated by the finding that mice deficient in this receptor develop hypertension in response to a high-sodium diet.

Unlike the changes in RIF PGE₂, the increases in RIF PGF₂α production were much smaller in response to a high-sodium diet than to a low-sodium diet. This is likely to reflect a reduced activity of the enzymes responsible for the conversion of PGE₂ to PGF₂α in view of the fact that this activity is known to be stimulated by Ang II via the AT₂ receptor. PGF₂α exerts a vasoconstrictor influence on the renal vasculature, in opposition to the vasodilatory effects of PGE₂. Therefore, a reduction in the conversion of PGE₂ to PGF₂α during high-sodium intake enables the natriuretic effects of PGE₂ to predominate, thus eliminating the increased sodium load. In adult rats, RIF PGF₂α production in response to high-sodium diet was significantly greater than that in young rats, suggesting that the conversion of PGE₂ to PGF₂α is downregulated to a lesser extent in adult rats than in young rats. An increased conversion of PGE₂ to PGF₂α in adult rats would also account for the decreased RIF PGE₂ levels observed in adult rats compared with young rats on a high-sodium diet. The mechanism underlying the increased conversion in adult rats is unclear, because the renin-angio-
tensin system was suppressed to a similar degree by a high-sodium diet in both young and adult rats; thus, Ang II levels would be very low in both groups. Nevertheless, these changes result in an increase in the PGF$_{2a}$-to-PGE$_2$ ratio in adult rats, which would be predicted to reduce the degree of natriuresis produced in response to the sodium load.

Overall, these results suggest that compared with the young kidney, the adult kidney has an altered response to high-sodium intake. In the young kidney, cGMP, cAMP, and PGE$_2$ production increase, and the ratio of PGF$_{2a}$ to PGE$_2$ decreases in response to sodium intake, all of which enable the kidney to eliminate the excess sodium load and thus maintain a constant total body sodium. Although the adult kidney also responds to high-sodium intake with an increase in cGMP, cAMP, and PGE$_2$, these increases are significantly decreased compared with those in the young kidney, and there is an increase in the ratio of PGF$_{2a}$ to PGE$_2$. Despite the alterations in the renal response to sodium loading in adult compared with young rats, we observed no difference in SBP between the 2 groups of rats, regardless of the level of sodium intake. This was in contrast to previous studies reporting that the blood pressure of 4-week-old Sprague-Dawley and Wistar-Kyoto rats is less than that of adult (10- to 12-week-old) rats.\textsuperscript{16,25}The reason for this discrepancy is unclear but may be related to the fact that the previous studies used anesthetized rats, whereas in the present study, blood pressure was measured in conscious rats.

In conclusion, the results of the present study demonstrate that compared with the young kidney, the adult kidney has an altered capacity to respond to stresses such as sodium loading. This may help to explain the phenomenon of declining renal function and increasing incidence of hypertension with age.

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