Cyclooxygenase-2 Mediates Increased Renal Renin Content Induced by Low-Sodium Diet

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Abstract We hypothesized that neuronal nitric oxide synthase and cyclooxygenase-2, which both exist in the renal cortex, predominantly in the macula densa, play a role in the control of renal renin tissue content. We studied the possible role of neuronal nitric oxide synthase in regulating renin tissue content by using mice in which the neuronal nitric oxide synthase gene has been disrupted (nNOS -/-) compared with its two progenitor strains, the 129/SvEv and the C57BL/6, to determine if the absence of neuronal nitric oxide synthase would result in decreased renin tissue content or blunt the increase observed during low sodium intake. Renal renin tissue content from cortical slices was determined in adult mice from all three strains maintained on a normal sodium diet. Renal renin content was significantly reduced in the nNOS -/- mice compared with the 129/SvEv and the C57BL/6 mice (3.1 ± 0.23 versus 5.66 ± 0.50 and 7.55 ± 1.17 μg angiotensin I/mg dry weight, respectively, P < 0.05), suggesting that neuronal nitric oxide synthase may stimulate renal renin content under basal conditions. Neither selective pharmacological inhibition of neuronal nitric oxide synthase using 7-nitroindazole or disruption of the neuronal nitric oxide synthase gene affected the increase in renal renin content observed during dietary sodium restriction. The influence of cyclooxygenase-2 on renal renin content through a macula densa-mediated pathway was studied using a selective cyclooxygenase-2 inhibitor. NS398, in 129/SvEv mice. A low-sodium diet increased renal renin content from 6.97 ± 0.52 to 11.59 ± 0.79 μg angiotensin I/mg dry weight (P < 0.005), but this increase was blocked by NS398. In addition, treatment with NS398 reduced renin mRNA in response to a low-sodium diet. Thus, increased renal renin content in response to dietary sodium restriction appears to require the induction of cyclooxygenase-2, while neuronal nitric oxide synthase appears to affect basal but not stimulated renal renin content (Hypertension. 1997;29[part 2]:297-302.)

Key Words • renin • cyclooxygenase • nitric oxide • mice

The mechanism by which a decreased sodium chloride delivery to the macula densa is sensed, resulting in increased renin secretion by the juxtaglomerular cells, remains unknown. However, since humoral factors are known to affect renin secretion and which has been implicated in renin regulation by both in vivo and in vitro studies, the enzyme NOS acts on its substrate L-arginine to produce NO. It exists in three known isoforms that are discretely localized throughout the nephron. Briggs et al demonstrated that the macula densa is rich in nNOS, whereas nNOS does not appear to be expressed in the glomerulus, mesangium, or juxtaglomerular cells. In contrast, eNOS is expressed in the glomerulus and blood vessels. Singh et al have also shown that the activity of nNOS in the macula densa is increased by a low-salt diet, whereas it does not affect ENOS. Therefore, NO produced in the macula densa could exert an effect directly on the adjoining juxtaglomerular cells or indirectly via other as yet unrecognized intermediates.

The role of NO in the regulation of renin is controversial. There have been studies showing that NO both stimulates and inhibits renin release. For example, studies using isolated juxtaglomerular cells in culture describe that NO inhibits renin release via its inhibitory second messenger, cGMP. Beierwaltes and Carretero have also reported that the NO donor, sodium nitroprusside, inhibits basal renin release from rat kidney cortical slices and that the nitric oxide synthase inhibitor L-NMMA has the opposite effect. In contrast, other investigators have demonstrated stimulatory effects of NO on renin release. Scholz and Kurtz reported that in the isolated kidney preparation, NO stimulated renin secretion, a result that is similar to that described in conscious dogs. Studies by He et al using the isolated perfused juxtaglomerular apparatus also describe increased renin when L-arginine was added directly to the luminal side of the macula densa and a reduction when the NOS inhibitor L-NAME was applied. However, the same authors also reported that application of an NO donor to the bath decreased renin secretion. Taken altogether, these results imply that renin secretion may be influenced differently by NO originating from at least two sources, tubular or macula densa derived versus that derived from the endothelial cells of the afferent arteriole.

Although most investigators agree that the direct inhibitory effect of NO on renin release is mediated by cGMP, a contradictory pathway by which NO could stimulate renin remains unclear. However, other autacoids, such as PGs, are known to stimulate renin. The prostacyclin (PGI2) is predominantly derived from the arteriolar endothelial cells and directly stimulates renin release from the juxtaglomerular cells, presumably through its second messenger, cAMP. On the other hand, PGE2 stimulation of renin requires the presence of an intact macula densa, and PGs have been implicated in the feedback regulation of renin release. Similar to...
NOS, the COX enzyme that releases PGs from arachidonic acid exists in both a constitutive form,\textsuperscript{16} COX-1, and inducible form,\textsuperscript{17} COX-2. Both isoforms have been localized in the kidney. Like nNOS in the renal cortex, COX-2 has been localized predominantly in the macula densa, and Harris et al.\textsuperscript{19} reported induction of COX-2 in the macula densa of rat kidney after dietary sodium restriction. This suggests a role for COX-2 in the regulation of salt, volume, and blood pressure homeostasis. Dietary sodium restriction increases both nNOS activity and induces COX-2 in the macula densa. Because of these similarities and because NO increases COX activity in other cell types,\textsuperscript{18} we hypothesized that an increase in nNOS, driven by dietary sodium restriction, is coupled to the induction of COX-2, resulting in the increase of renin through products of arachidonic acid metabolism.

**Methods**

Previous studies looking at nNOS and renin have been compromised by the lack of specificity in their pharmacological tools. However, the development of selective inhibitors of nNOS as well as the development of transgenic mice that have had the gene encoding for nNOS “disrupted” (nNOS \textsuperscript{--/--} mice) provides more specific experimental tools.\textsuperscript{20} Thus, the use of these mice in this study and comparisons with both progenitor strains or with mice that have been treated with N-nitro-L-arginine methyl ester (L-NAME) provide an assessment of the role of macula densa-derived NO in the regulation of renin. Likewise, the formulation of specific COX-2 inhibitors has enabled us to examine the role of this enzyme in the macula densa mechanism for the regulation of renin.

This study examines renal renin content in nNOS \textsuperscript{--/--} mice and its progenitor strains, the 129/SvEv and C57BL/6 mice. Unlike humans or rats, certain strains of mice have significant extrarenal renin from sites such as the submandibular gland, which may affect plasma renin concentrations.\textsuperscript{22} Hence, plasma renin concentrations in mice do not accurately reflect renin release specifically from the kidney. Mouse plasma renin values are subject to wide variability and do not respond to stimuli known to stimulate renin, eg, a low-salt diet. Our own studies have found high circulating plasma renin concentrations that were not different between 129/SvEv control mice (0.35±0.09 μg Ang I mL\textsuperscript{-1} h\textsuperscript{-1}) and nNOS \textsuperscript{--/--} mice (0.47±0.19 μg Ang I mL\textsuperscript{-1} h\textsuperscript{-1}). Thus, we have focused our study on renal renin content. We examined the effect of selective nNOS inhibition or nNOS gene disruption on renal renin content in response to a chronic low-salt diet, a stimulus known to stimulate renin through the macula densa. Finally, we examined the effect of COX-2 inhibition on the stimulation of renal renin content exerted by dietary sodium restriction.

**Animals**

Experiments were performed using 129/SvEv, C57BL/6, and nNOS \textsuperscript{--/--} mice. 129/SvEv and C57BL/6 mice were obtained from Taconic Farms (Germantown, NY) and nNOS \textsuperscript{--/--} mice breeding pairs were provided by Drs Paul Huang and Mark Fishman, Massachusetts General Hospital. These breeding pairs were generated from initial nNOS \textsuperscript{+/--} hybrids of C57BL/6 and 129/Sv strains from which nNOS \textsuperscript{--/--} offspring were obtained. They were subsequently interbred and maintained as obligate nNOS \textsuperscript{--/--} mice for an unspecified number of generations. From these parents, homozygous nNOS \textsuperscript{--/--} mice were bred in our own animal facility. Unless otherwise specified, all mice were maintained on a normal diet and were allowed access to water ad libitum. All experiments were carried out within the guidelines of the Institutional Animal Care and Use Committee of Henry Ford Hospital.

**Renal Renin Content**

Renal renin content was determined by measuring the Ang I-generating capacity of homogenized renal cortical slices. Briefly, mice were anesthetized with sodium pentobarbital and their kidneys removed and placed immediately into ice-cold MEM (composition. 125 mmol/L NaCl, 19 mmol/L NaHCO\textsubscript{3}, 4 mmol/L KCl, 2.6 mmol/L CaCl\textsubscript{2}.12 mmol/L NaHPO\textsubscript{4}, 0.8 mmol/L MgSO\textsubscript{4}.2 g/mL glucose, oxygenated to pH 7.4). The kidneys were rinsed in cold MEM and decapsulated, and one slice of superficial cortex was cut from each lateral surface using a Stadie-Riggs microtome. The two slices were blotted and weighed, homogenized in 1 mL buffer, and centrifuged at 2000 rpm for 20 minutes, and the supernatant was removed. For the generation of Ang I, 1.50 dilutions of the supernatant were made with MEM buffer, then 5-μL aliquots were taken and diluted aluminum with 95-μL buffer. A 200-μL aliquot of a peptide inhibitor cocktail (3% PMSF in methanol and 3% EDTA, pH 6.5) and 200 μL sheep angiotensinogen (500 ng) were then added, and the total mixture was incubated at 37°C for 30 minutes. To terminate the reaction, samples were placed on ice and 500 μL of 0.015 mol/L HCl was added. Tubes were boiled for 10 minutes and centrifuged at 2000 rpm for 20 minutes, and the supernatants were removed. Generated Ang I was measured by radioimmunoassay using methods previously published,\textsuperscript{23} and the results are expressed as milligrams of Ang I per milligram of dry weight per hour. Dry weight was calculated to be 14% of wet weight based on data obtained from previous experiments. Experiments designed to test the validity of this assay have demonstrated that less than 10% of the substrate is consumed under these conditions and have shown linearity of product generated with time. Nephrectomized sheep angiotensinogen was used as a substrate since it is known that mouse renin can release Ang I from this heterologous substrate.

**RNA Isolation and Analysis**

Expression of renin mRNA was determined from two additional groups of mice that had been placed on either a low-salt diet alone or a low-salt diet plus the COX-2 inhibitor. At the end of the experimental period, the kidneys were removed and placed into ice-cold phosphate-buffered saline. Two cortical slices were taken from each surface of the kidney, and the four slices from each mouse were used for the preparation of total RNA. Briefly, slices were homogenized in 1 mL RNA Stat (Tel-Test B) and after ethanol precipitation, RNA pellets were resuspended in 25 μL water. Twenty micrograms total RNA was denatured with dimethyl sulfoxide and glyoxal, separated on a 1% agarose gel, and blotted onto a nylon membrane (Gibco BRL). Rennin mRNA was detected using a 1430-bp cDNA probe (kindly provided by Dr K. Lynch, University of Virginia) and normalized to 18S as a loading control. Hybridization was performed at 60°C using renin cDNA as a probe and at 42°C using an 18S oligonucleotide probe. Washing conditions are as described 2X SSC, two times for 15 minutes at room temperature; 0.1 X SSC, two times for 15 minutes at room temperature, 0.1 X SSC, two times for 15 minutes at 60°C. Nylon filters were exposed to Kodak X-Omat AR film for 1 to 5 days. Densitometry (model GS-670, Bio-Rad) was used to quantitate mRNA and was normalized to 18S as a loading control. Light exposures were used for densitometry whereas darker exposures were used to make Fig 4.
Determination of Systolic Blood Pressure

Systolic blood pressure was determined using a newly designed and validated noninvasive computerized tail-cuff system (BP-2000, Visitech Systems). Mice were first trained for 7 days, then measurements were recorded daily on 5 consecutive days. Each session included two sets of 10 measurements for a total of 10. In order to include each set of measurements for an individual mouse, the computer had to successfully identify a blood pressure in at least 6 of the 10 trials within a set.

Statistical Analysis

To examine differences between the three strains of mice, the data were transformed using natural logarithms because of unequal variances. Dunnett’s test was then applied to the transformed data and values of P< 0.05 were considered significant. For all other analyses, log transformation was also applied and data were analyzed by Student’s t test with a value of P<0.05 considered significant.

Protocols

Comparison of Renal Renin Content in nNOS −/− Mice With C57BL/6 and 129/SvEv Mice

Since the nNOS −/− mouse is derived from two different progenitor strains, the 129/SvEv and the C57BL/6 mouse, both of these strains were initially used as controls. Male mice from all three strains weighing 25 to 30 g were maintained on a normal sodium diet and were allowed access to water ad libitum. Mice were anesthetized with sodium pentobarbital and their kidneys removed for determination of renal renin content. The number of mice studied for each strain was as follows: 129/SvEv, n=11, C57BL/6, n=10, and nNOS −/−, n=9. Because blood pressure and the renin-angiotensin system are so closely interdependent, systolic blood pressures were also measured from all three strains of mice by the tail-cuff method.

Effect of Dietary Sodium Restriction on Renal Renin Content in Mice Deficient in nNOS

To determine whether genetic disruption of the nNOS gene altered the increase in renin content by dietary sodium restriction, experiments were performed using nNOS −/− mice on a low-sodium diet. Male nNOS −/− mice were placed on a diet containing 0.02% sodium for 14 days (n=16), then the kidneys were removed and cortical slices harvested to determine renal renin content. These values were compared with those from nNOS −/− mice maintained on a normal sodium diet (n=9).

Effect of Chronic 7-NI on Renal Renin Content Stimulated by Dietary Sodium Restriction

We examined the effect of chronic treatment with the selective nNOS inhibitor 7-NI on renal renin content of 129/SvEv mice. Male 129/SvEv mice were placed on either a 0.02% low-sodium diet or a 0.4% normal sodium diet (Purina Mills) for 14 days. Mice from each group received either 7-NI (20 mg·kg⁻¹·d⁻¹ IP) or peanut oil vehicle during this period. On the last day, the mice were euthanized and the kidneys removed to obtain cortical slices and determine renal renin content. The number of mice in each group is given in parentheses after each group description: low sodium plus vehicle (12), low sodium plus 7-NI (15), normal sodium plus vehicle (14), and normal sodium plus 7-NI (16).

Effect of COX-2 Inhibition on Renal Renin Content Stimulated by Dietary Sodium Restriction and on Renin mRNA Expression

To determine whether COX-2 plays a role in the increase in renal renin content in response to dietary sodium restriction, control 129/SvEv mice were placed on a low-sodium diet for 14 days. During this period, each diet group received either vehicle or 1 mg·kg⁻¹·d⁻¹ of a selective COX-2 inhibitor, NS 398 (Cayman Chemical Co.), in the drinking water. On the final day, the kidneys were removed to determine renal renin content of cortical slices. The number of mice in each group was as follows: normal sodium plus vehicle, n=14, low sodium plus vehicle, n=12; and low sodium plus NS398, n=19. Two additional groups of mice were placed on either a low-sodium diet or a low-sodium diet plus NS398, as above. These mice were used for determination of systolic blood pressure and to measure expression of renin mRNA in the renal cortex.

Although we did not anticipate that inhibition of COX-2 would have any effect under conditions in which the enzyme was not induced, we subsequently ran an additional control in which two groups of mice were paired and placed on either a normal sodium diet (n=10) or a normal sodium plus NS398 (n=10) for 2 weeks, as before. The kidneys were then harvested for determination of renal renin content, as above.

Results

Comparison of Renal Renin Content in C57BL/6, 129/SvEv, and nNOS −/− Mice

As shown in Fig 1, renal renin content from the nNOS −/− mice (3.11±0.23 μg Ang I·mg⁻¹·h⁻¹) was 45% less than cortical renin content in 129/SvEv mice (5.60±0.50 μg Ang I·mg⁻¹·h⁻¹, P<0.005) and 59% less than in C57BL/6 mice (7.55±1.17 μg Ang I·mg⁻¹·h⁻¹, P<0.005). Renal renin content was not significantly different between the two control groups. Systolic blood pressures were similar in the 129/SvEv and nNOS −/− mice (116±2.3 and 110±2.7 mm Hg, respectively) but were reduced in the C57BL/6 mice (96±3.3 mm Hg). Because we were concerned about the impact of lower renal perfusion on renin content in the C57BL/6 mice, only 129/SvEv mice were used as a control strain in subsequent experiments.

Effect of Dietary Sodium Restriction on Renal Renin Content in Mice Deficient in nNOS

While nNOS −/− mice exhibited a 45% reduction in renal renin content compared with their control strains (Fig 1), they were able to respond to chronic dietary sodium restriction by increasing renal renin content twofold (Fig 2), from 3.11±0.23 to 6.54±0.40 μg Ang I·mg⁻¹·h⁻¹ (P<0.005). This increase is of a similar magnitude as the increase in renal renin content observed in control 129/SvEv mice fed a low-sodium diet.
Effect of Chronic 7-NI on Renal Renin Content Stimulated by Dietary Sodium Restriction

In 129/SvEv mice, renal renin content (Fig 2) was increased nearly twofold by 14 days' dietary sodium restriction to 11.59±0.79 μg Ang I·mg⁻¹·h⁻¹ compared with renal renin content on a normal sodium diet (6.97±0.53 μg Ang I·mg⁻¹·h⁻¹; P<.005). Chronic 7-NI had no significant influence on renal renin content from mice on either diet. Renal renin content was 6.24±0.51 μg Ang I·mg⁻¹·h⁻¹ for mice on a normal sodium diet receiving 7-NI and 9.53±0.97 μg Ang I·mg⁻¹·h⁻¹ for mice on a low-sodium diet receiving 7-NI.

Effect of COX-2 Inhibition on Renal Renin Content and Renin mRNA Stimulated by Dietary Sodium Restriction

In 129/SvEv mice maintained on a low-sodium diet for 14 days, renal renin content was 11.59±0.79 μg Ang I·mg⁻¹·h⁻¹, whereas concurrent treatment of mice on a low-sodium diet with the COX-2 inhibitor NS398 reduced renal renin content (Fig 3) by 39%, to a value of 7.11±0.72 μg Ang I·mg⁻¹·h⁻¹ (P<.005), which was similar to that observed in mice on a normal sodium diet (6.97±0.52 μg Ang I·mg⁻¹·h⁻¹). In addition, inhibition of COX-2 reduced renin mRNA by 60% in mice on a low-sodium diet (Fig 4). Densitometric units (corrected to 18S) for renin mRNA were 1.9±0.07 on a low-sodium diet alone (n=3 mice) and 0.73±0.24 (n=5 mice) for the low-sodium diet plus NS398, P<.02. In contrast, in additional paired studies, renal renin content in mice on a normal sodium diet was the same as for mice on a normal sodium diet given NS398 (3.19±0.47 μg Ang I·mg⁻¹·h⁻¹ versus 3.44±0.28 μg Ang I·mg⁻¹·h⁻¹, respectively). The renin values here were reduced compared with our original normal sodium diet controls (above). Why renin content was lower in these mice is unclear, though many factors may influence basal renin. However, the NS398-treated mice were paired with their untreated controls. Since the treatment of paired animals with NS398 did not affect renal renin content, this suggests that inhibition of COX-2 has no measurable effect under basal conditions. We also observed that chronic COX-2 inhibition did not affect systolic blood pressure, which was 109±2 mm Hg in mice on a low-sodium diet and 113±2 mm Hg in mice on the low-sodium diet plus NS398 (n=5 mice per group).

Discussion

The major finding of this study is that the increase in renal renin content in response to a low-sodium diet, presumably through the macula densa, appears to depend on
the induction of COX-2. In addition, although chronic inhibition of nNOS does not seem to alter the change in renal renin content elicited by dietary sodium restriction, we observed a reduced renin content in mice in which the nNOS synthase gene was disrupted. Thus, while nNOS may influence basal renin content in the mouse, it does not seem to be critical in the stimulation of renin content by chronic dietary sodium restriction.

Despite the development of transgenic animal models, there is a lack of current literature regarding normal mouse physiology and, in particular, of the regulation of the renin–angiotensin pathway in this species. Moreover, any such information is generally limited to plasma or circulating renin concentrations, which may be greatly affected by extrarenal renin. In particular, certain strains of mice have large amounts of submandibular renin,22 and we have found that PRC values in mice exhibit great variability. Additionally, our own studies demonstrate that although dietary sodium restriction increases renal renin content, it does not alter PRC in mice. Hence, it is unlikely that plasma values accurately reflect renin released from the kidneys. This study has measured renal renin content, which is a balance between synthesis and release, to more accurately reflect renin in the kidney.

Initial experiments compared renal renin content of cortical slices in the nNOS −/− mouse with those in its two progenitor strains, the 129/SvEv and the C57BL/6. Surprisingly, renal renin content was reduced in the nNOS −/− mice compared with either control strain. The lack of difference between control strains was not unexpected even though the C57BL/6 mouse has only one copy of the renin gene compared with the two copies in the 129/SvEv. The Ren-2 gene present in the 129/SvEv strain is believed to be responsible for production of renin from extrarenal sites such as the submandibular gland and hence, its presence might not be expected to alter renin content of the kidney. Since studies in our laboratory have demonstrated that blood pressure is similar in the nNOS −/− and 129/SvEv strains, it could be concluded that the reduced renin content in the nNOS −/− mice was not related to altered renal baroreceptor control secondary to altered renal perfusion. However, it may be a direct effect, related to the absence of nNOS in the macula densa or perhaps secondary to possible increased sympathetic drive in this strain of mouse (X.-P. Yang, unpublished observations, 1996).

To investigate the role of nNOS in macula densa-mediated changes in renin, we examined the effect of dietary sodium restriction on renal renin content in both nNOS −/− mice and control mice treated chronically with the selective nNOS inhibitor 7-NI. Similar to the rat, dietary sodium restriction for 14 days increased renal renin content in 129/SvEv mice by 66%. Both nNOS −/− and mice treated chronically with 7-NI were able to respond to sodium restriction by an increase in renal renin content. These results contrast with our original hypothesis and with previous studies by Reid and Chou26 and by Bierwaltes,20 who found that nNOS inhibition blocked renin stimulated by furosemide, a stimulus mediated through the macula densa. Whether these results reflect species differences is unclear. In the rat, renal renin content parallels the increase in plasma levels. In the mouse, we have found that PRC does not correlate with renal renin content because plasma values are affected by extrarenal renin, and we have not measured renin secretion rates. Thus, the difference between the present results and those in studies in the rabbit29 and in the rat30 is that they measured plasma renin in response to acute stimulation whereas we have studied chronic adaptation of renal renin content. In addition, although dietary sodium restriction is widely regarded as a stimulus for macula densa-mediated renin secretion, we are aware that ingestion of a low-sodium diet may influence other stimuli and sympathetic activity, all of which could affect renin and whose interaction with nNOS we cannot predict. However, we did find that either nNOS −/− mice or mice treated chronically with 7-NI still respond to dietary sodium restriction by increasing renin content. If this stimulus acts through the macula densa, as hypothesized, it implies a diminished role for NO in this particular pathway to increase renin content. However, the fact that basal renal renin content was reduced in the nNOS −/− mice suggests that it would be prudent to examine the involvement of nNOS in other mechanisms controlling renin.

In the current literature, there is one report indicating that the nNOS −/− mouse may not be a true null mutation and that some residual nNOS activity remains in certain tissues, although it is greatly reduced. This study indicated that residual but greatly depressed NOS catalytic activity was detected in the brain. It did not examine whether any activity remains in the kidney. Thus, it is possible that some residual nNOS could contribute to the increase in renal renin content noted in nNOS −/− mice when placed on a low-sodium diet. Although we cannot rule out this possibility, the extent to which renin increases after dietary sodium restriction would seem incompatible with such a small residual amount of nNOS in the kidney, if it exists. Furthermore, the fact that we found that both disruption of the nNOS gene and pharmacological inhibition of nNOS with 7-NI had the same effect (ie, did not modify the response to dietary sodium restriction) further suggests that nNOS does not participate in this pathway to increase tissue renin content.

Treatment with the selective COX-2 inhibitor NS398 abolished the rise in renin content stimulated by 14 days' dietary sodium restriction, and we also found that it reduced renin mRNA. These results are the first to suggest that selective COX-2 inhibition reduces renin synthesis and provide clear evidence that COX-2 plays a significant role in the increase of renal renin content in response to dietary sodium restriction. Importantly, the renin response seems to require COX-2 induction by sodium restriction,18 as the inhibitor had no effect on renin content in kidneys of mice on a normal sodium diet. There is evidence in the rat from earlier studies that demonstrate the increase in plasma renin activity in response to a low-sodium diet is dependent on PG synthesis and can be prevented by treatment with indomethacin,32 which would inhibit both COX-1 and COX-2. Although the present study did not determine which prostanoid might be responsible for the increase in renal renin content in response to reduced sodium, one may speculate that PGE2 might be responsible since its effects have already been shown to require the presence of an intact macula densa19 and it stimulates renin. However, we did not measure PGE2 in the present study. Although COX-1 and COX-2 are 60% homologous in their amino acid sequence,33 no study has presented evidence as to the profile of prostanoids produced by the different isoenzymes. Likewise, the renal hemodynamic responses to COX-2 inhibition in the mouse...
have not been evaluated, though studies in the rat suggest that it does not change renal blood flow.24 Our study found that chronic treatment with NS398 does not alter systemic blood pressure of mice on a low-sodium diet. Thus, the changes in renal renin content observed in our study do not appear to be secondary to alterations in renal hemodynamics.

In conclusion, this study has demonstrated that COX-2 is a critical component of the pathway leading to increased renal renin content in response to dietary sodium restriction in the mouse, presumably mediated through the macula densa. We did not find any strong evidence that neuronal nitric oxide plays a role in mediating the increase in renal renin content under these conditions in the mouse. While the precise pathway by which COX-2 is associated with increased renin content is not yet clear, it does appear to be a critical and significant step in the renin response to dietary sodium restriction.

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