Inhibition of macula densa-stimulated renin secretion by pharmacological blockade of cyclooxygenase-2

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Traynor, Timothy R., Ann Smart, Josephine P. Briggs, and Jurgen Schnermann. Inhibition of macula densa-stimulated renin secretion by pharmacological blockade of cyclooxygenase-2. Am. J. Physiol. 277 (Renal Physiol. 46): F706–F710, 1999.—Previous results from our laboratory have shown that in the isolated perfused juxtaglomerular apparatus, nonselective inhibitors of cyclooxygenase (COX) activity prevent the stimulation of renin secretion by a reduction in luminal NaCl concentration at the macula densa. The present studies were performed to examine which COX isoform is involved in NaCl-dependent renin secretion. In the absence of COX inhibitors, a reduction in luminal NaCl (from Na 141/Cl 120 mM to Na 26/Cl 7 mM) caused an increase in renin secretion rate from 4.5 ± 1.8 to 26.1 ± 7.4 nGU/min (P < 0.01, n = 19). The presence of the COX-1 inhibitor valerylsalicylate (500 µM) in lumen and bath did not affect the stimulation of renin secretion by a reduction in luminal NaCl concentration (5 ± 1.8 nGU/min at high NaCl, and 30.5 ± 9.4 nGU/min at low NaCl; P < 0.01, n = 8). In contrast, the specific COX-2 inhibitor NS-398 (50 µM) in lumen and bath abolished the stimulating effect of low luminal NaCl (12.8 ± 3.9 nGU/min at high NaCl, and 10.7 ± 3.1 nGU/min at low NaCl; NS, n = 15). The finding that COX-2 is critically involved in macula densa control of renin secretion indicates that the COX-2-expressing epithelial cells in the tubuloglomerular contact area are a likely source of prostaglandins participating in the signaling pathway between the macula densa and renin-producing granular cells.

Kidney: rabbit; NS-398; valerylsalicylate; isolated perfused juxtaglomerular apparatus

The ability of prostaglandins to stimulate renin secretion was first demonstrated in experiments in the anesthetized rabbit in which arachidonic acid infusion was observed to increase plasma renin activity and indomethacin to decrease it (28). Subsequently, prostaglandins, specifically PGE2 and PGJ2, have been found to augment renin secretion in numerous in vitro preparations including renal cortical tissue slices, isolated glomeruli, and isolated nonperfused afferent arterioles (2, 10, 22, 45). Most recently, stimulation of renin release by PGE2 has been observed in primary cultures of juxtaglomerular granular cells providing direct evidence for the notion that prostaglandins can directly interact with renin-generating cells (24). Substantial experimental effort has also been directed toward elucidating the possible involvement of prostanoids in any of the known physiological control mechanisms of renin secretion. Whereas the evidence in support of a participation of prostaglandin formation in baroreceptor-mediated renin secretion is ambiguous, results from various experimental approaches have suggested a role for prostaglandins in the macula densa mechanism for control of renin secretion (7). For example, the renin secretory response to furosemide or dietary sodium restriction was found to be diminished during inhibition of prostaglandin synthesis (9). Furthermore, the stimulation of renin secretion by a reduction in renal perfusion pressure under experimental conditions that minimize a mediating role of baroreceptors was found to be indomethacin sensitive (12).

Using the isolated perfused juxtaglomerular apparatus (JGA), Greenberg et al. (13) demonstrated that inhibition of cyclooxygenase (COX) with flurbiprofen or flufenamic acid abolished the stimulatory effect of a decrease in macula densa NaCl concentration on renin release. These observations confirmed the critical role of prostaglandins in macula densa control of renin secretion and established the local character of the COX-PG system in this regulation. Earlier immunocytochemical evidence had shown the presence of COX in mesangial cells but not in the macula densa, suggesting that the origin of prostaglandins involved in macula densa control of renin release was the glomerular or extraglomerular mesangium (42). However, the discovery of a second COX isoform, COX-2, and the demonstration that in the renal cortex expression of COX-2 is restricted to the macula densa and to cells of the thick ascending limb (TAL) near the macula densa has raised the possibility that the epithelium in the tubuloglomerular contact area may be an alternative source of prostaglandins in the macula densa mechanism for control of renin secretion (7). For example, the renin secretory response to furosemide or dietary sodium restriction was found to be diminished during inhibition of prostaglandin synthesis (9). Furthermore, the stimulation of renin secretion by a reduction in renal perfusion pressure under experimental conditions that minimize a mediating role of baroreceptors was found to be indomethacin sensitive (12).

The development of inhibitors with relative selectivity for COX-1 or COX-2, such as valerylsalicylate and NS-398, has made it possible to pharmacologically assess the function of the two COX isoforms separately (4, 11). In the present experiments, we have compared the effects of valerylsalicylate and NS-398 on the stimulation of renin secretion by a reduction in luminal NaCl concentration in the isolated perfused JGA preparation. Our data show that interference with COX-2, but not with COX-1, is associated with marked inhibition of the renin stimulatory effect of a low luminal NaCl concentration. These results provide evidence to indicate that prostaglandins generated by COX-2 in macula densa cells play an important role in the
METHODS

Experiments were performed using specimens from female New Zealand White rabbits in a body weight range between 0.6 and 1.3 kg. The animals were maintained on a standard rabbit chow. After the animals were killed, kidneys were decapsulated, cut into coronal sections and placed in ice-cold DMEM with Ham's nutrient mixture F-12 (Sigma Chemical, St. Louis, MO) containing 3% fetal calf serum (Life Technologies). Immediately before use, the dissection medium was aerated with 95% O2-5% CO2 and its pH was adjusted to 7.4.

Specimens were dissected and perfused as previously described (41). Briefly, the macula densa segment with a short portion of the TAL, early distal convoluted tubule (DCT), adherent glomerulus, and short fragments of arterioles were dissected from the cortical portion of medullary rays. The specimen was transferred into a thermoregulated bath chamber (38°C) mounted on the stage of an inverted microscope (Olympus, Overland Park, KS), and the tubule was cannulated, usually from the TAL although occasionally from the DCT. The tubule was perfused using a system of three pipettes: a perfusion pipette, a holding pipette, and a superfusion pipette. This perfusion setup allowed for the exchange of the perfusate during the course of the experiment. Perfusion flow was maintained at ~50 nl/min using a hydraulic pressure head. After cannulation, the superfusion pipette was advanced to cover the perfused specimen, and superfusion with DMEM containing 3% bovine serum albumin (Sigma Chemical) was started at a rate of ~2 µl/min. The bath medium was exchanged with water-equilibrated mineral oil, and the specimen was allowed to equilibrate for 15–20 min. At 8-min intervals, the droplet forming at the tip of the superfusion pipette was collected using a tapered microcapillary tube. The collected sample was frozen for later determination of renin activity.

Experiments consisted of three periods, each with four 8-min superfusate collections. During the first period, specimens were perfused with an isotonic NaCl solution (141 mM Na, 120 mM Cl) consisting of (in mM) 115 NaCl, 25 NaHCO3, 0.96 NaH2PO4, 0.24 Na2HPO4, 5 KCl, 1.2 MgSO4, 1 CaCl2, and 5.5 glucose, pH 7.4 and aerated with 95% O2-5% CO2. In the second period the perfusate was exchanged for a solution in which NaCl concentration was reduced by 115 mM to 26 mM Na and 7 mM Cl. For the final period, the specimen was again perfused with the isotonic NaCl solution. Perfusion rate and efficiency of perfusate exchange were determined by adding [3H]inulin to the low-NaCl perfusate and measuring the rate of appearance of beta activity in a 3-min sample collected at the end of each period. In experiments testing prostaglandin synthesis inhibition specimens were treated with either 500 µM valerylsalicylate or 50 µM NS-398 (Cayman Chemical, Ann Arbor, M1) in both the perfusate and bath solutions 70–80 min before reducing luminal NaCl concentration.

Renin content of each superfusate sample was determined by radioimmunoassay of generated angiotensin I using the antibody trapping technique (31). Renin values were standardized by reference to renin standards obtained from the Institute for Medical Research (MRC, Holly Hill, London, UK), and the values are expressed in Goldblatt hog units (GU). Differences between mean values were determined using a paired t-test with P < 0.05 considered significant.

RESULTS

Stimulation of the macula densa mechanism by a step decrease in NaCl concentration resulted in a sustained increase in renin secretion that was fully reversible when the perfusate was returned to a high-NaCl solution (Fig. 1, top). As shown in Fig. 2, renin secretion rate in the initial high-NaCl period averaged 4.5 ± 1.8 nGU/min. During perfusion with the low-NaCl solution (period 2), renin secretion increased to a mean value of 26.1 ± 7.4 nGU/min (P < 0.01, n = 19). Return to the high-NaCl solution (period 3) caused average renin secretion to fall to 10.4 ± 4 nGU/min, a level not significantly different from that in the initial period. The nearly sixfold increase in renin secretion in response to a reduction in NaCl in this preparation is comparable to that observed in previous studies from this laboratory (13, 30, 41).

As shown in Fig. 1 (middle) stimulation of renin secretion by low NaCl was not affected by the addition of valerylsalicylate at 500 µM, a concentration that has been shown to be maximally selective for inhibition of COX-1 (4). In the presence of valerylsalicylate, renin secretion averaged 5 ± 1.8 nGU/min in the initial high-NaCl period, 30.5 ± 9.4 nGU/min during the low-NaCl perfusion period (P < 0.01, n = 8), and 11.6 ± 3.7 nGU/min during the final high-NaCl period (Fig. 2).

![Fig. 1. Time course of changes in renin secretion rate in perfused juxtaglomerular apparatus specimens in response to a decrease in luminal NaCl concentration from 141 mM Na/120 mM Cl (high NaCl) to 26 mM Na/7 mM Cl (low NaCl) and back to high luminal NaCl. Top: control (n = 19). Middle: in presence of the cyclooxygenase-1 (COX-1) inhibitor valerylsalicylate at 500 µM (n = 8), bottom: in presence of the COX-2 inhibitor NS-398 at 50 µM (n = 15).](http://ajprenal.physiology.org/)

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- **Methods**: Techniques and protocols used in the experiments.
- **Results**: Findings and outcomes of the experiments.
- **Discussion**: Potential implications and further research considerations.
- **Conclusion**: Summary of the research and its significance.
Macula densa-mediated stimulation of renin secretion in the isolated perfused JGA preparation was found to be abolished in the presence of 50 µM NS-398, a specific inhibitor of COX-2 (Fig. 1, bottom). On average, renin secretion was 12.8 ± 3.9 nG U/min during high-NaCl perfusion, 10.7 ± 3.1 nG U/min during low-NaCl perfusion, and 10.9 ± 3.8 nG U/min during the final high-NaCl period (n = 15; Fig. 2).

**DISCUSSION**

The current studies extend previous investigations from our and other laboratories that sought to clarify the role of prostaglandins in macula densa-controlled renin secretion. Using the isolated perfused JGA preparation, we previously found that nonselective inhibitors of cyclooxygenases almost completely abolished the stimulatory effect of a reduction in luminal NaCl concentration on renin secretion (13). These data fully corroborated earlier, less direct evidence to suggest that macula densa-mediated renin release is prostaglandin dependent (9, 12, 29). Although establishing an involvement of prostaglandins in macula densa control of renin secretion, these experiments did not resolve the question of their cellular source and of the identity of the COX isoform responsible for their generation.

Results from previous studies had suggested that prostaglandins produced by mesangial cells were primarily responsible for NaCl-dependent regulation of renin release (27, 30). Immunocytochemical evidence indicated that COX was localized to mesangial cells, endothelial cells of renal vessels, and parietal cells of Bowman's capsule (42). Mesangial cells had also been shown to be able to convert exogenous arachidonic acid to prostaglandins (1). It was therefore speculated that prostaglandins generated within the extraglomerular mesangium were involved in the juxtaglomerular signaling pathway. Furthermore, prostaglandin production by cultured mesangial cells was found to be stimulated by a reduction in extracellular chloride concentration (35). This dependency was suggested to possibly act as a coupling mechanism between the luminal NaCl signal and the mesangial prostaglandin response on the assumption that chloride concentration in the mesangial interstitium changes in parallel to luminal chloride (27).

However, it was recently discovered that COX-2, an isoform typically induced by inflammatory cytokines, is constitutively expressed in macula densa cells and in surrounding TAL cells (15, 17, 18, 46). This finding raised the possibility that macula densa cells are the source of prostaglandins involved in juxtaglomerular cell-to-cell signaling. In the present experiments, we have investigated which of the two COX isoforms, and by implication which cell type, is involved in macula densa control of renin secretion by using isoform-specific inhibitors of COX activity. Our data show that NS-398, a COX-2-specific inhibitor, prevented NaCl-dependent changes in renin secretion, whereas valeryl salicylate, a COX-1 inhibitor, had no effect. NS-398 has been shown to inhibit the activity of purified COX-2 with an IC$_{50}$ value of ~1 µM, whereas it did not affect COX-1 activity up to a concentration of 100 µM (11). Thus it appears that this compound at the 50 µM concentration used in our studies can be assumed to selectively and completely inhibit COX-2 activity. Compared with NS-398, valeryl salicylate has a narrower window of selectivity, with the IC$_{50}$ value for inhibition of COX-1 exceeding that for COX-2 only by a factor of ~20 (4).

The finding of inhibition of macula densa-dependent renin secretion by COX-2 blockade advances the understanding of cell-to-cell signaling mechanisms in the JGA by localizing the origin of the prostaglandins involved in the renin regulatory pathway to epithelial cells in the tubuloglomerular contact area. This conclusion is based on in situ hybridization and immunohistochemical evidence showing rather consistent expression of COX-2 in macula densa cells and occasionally in TAL cells in their vicinity (15, 17, 18). Under nonstimulated conditions, COX-2 expression was not detected in any other location in the renal cortex. Specifically, constitutive COX-2 expression was not detected in association with mesangial cells, a cell type that expresses COX-1 and can respond to cytokines with strong induction of COX-2 (37). Our data suggest that COX-2 in macula densa cells and/or adjacent cortical TAL cells is a required participant in the pathway that couples the tubular epithelium with the renin producing granular cells.

It is still unclear how changes in luminal NaCl concentration affect macula densa prostaglandin production, but several possibilities exist. It has been shown recently that macula densa cells express a cytosolic phospholipase A$_2$ (cPLA$_2$), which is activated by calcium in the 10$^{-7}$ M concentration range (14, 32). Thus, if a reduction in luminal NaCl causes cytosolic Ca$^{2+}$ in macula densa cells to increase, then cPLA$_2$ activation and subsequent release of arachidonic acid from a membrane pool could be responsible for enhanced prostaglandin production. The evidence for an inverse relationship between luminal NaCl and macula densa cytosolic calcium, however, is not fully convincing, since the opposite, a direct relationship, has also been reported (3, 38). An alternative possibility would be that...
the effects of a reduction in luminal NaCl are mediated directly by changes in the activity of macula densa COX-2. Regulation of COX-2 activity is not fully understood, but one possibility would be that the COX-2 activation is a consequence of increased NO production by the neuronal NO synthase in macula densa cells (33). There is substantial evidence to indicate that NO is an activator of COX-2 in a number of different tissues (8, 20, 21, 36, 39). Conceivably, therefore, the rate of NO production in macula densa cells presumably through the activation of neuronal nitric oxide synthase (nNOS) may be a determinant of COX-2 activity in the same cells. Our previous observation in the same JGA GA preparation that the presence of a NOS inhibitor in the tubular perfusate significantly reduces the stimulation of renin secretion by low NaCl is consistent with this possibility (19).

Prostaglandins released by epithelial cells in the juxtaglomerular region may act as diffusible mediators between macula densa and granular cells. Currently available evidence does not establish whether mesangial cells or granular cells are the targets for any released COX-2 products. However, it would seem conceivable that PGE$_2$ and/or PGF$_2\alpha$ interact directly with the renin-producing cells. The demonstration that both prostaglandins can stimulate renin release from isolated cells asserts functionally that prostaglandin receptors are present in these cells (24). The mRNAs for PGE$_2$ receptors of the EP$_4$ and possibly EP$_2$ subtypes have been found to be expressed abundantly in glomeruli of human, rabbit, and mouse kidneys, even though their cellular association has not been established with certainty (5, 6, 43). Similarly, the presence of prostacyclin (IP) receptors in renal vascular and glomerular cells has been documented by immunocytochemistry and in situ hybridization, although juxtaglomerular granular cells may not be included in the IP receptor-expressing cells (26, 34). PGE$_2$ receptors of the EP$_4$/EP$_2$ subtype are G$_s$ coupled and cause an activation of adenylyl cyclase (6). It is consistent with this signal transduction mechanism that PGE$_2$ and PGF$_2\alpha$ have been shown to cause an increase in granular cell cAMP (24). This observation agrees well with the consistent and powerful action of cAMP to mediate stimulation of renin secretion as well as renin mRNA expression (25, 44). Nevertheless, an effect of prostaglandins on extraglomerular mesangial cells cannot be ruled out.

A perspective of the relationship between luminal NaCl, macula densa COX-2, and granular cell renin that was not addressed in the present studies, but deserves further investigation, is the possibility that a persistent decrease in luminal NaCl may cause a causally linked induction of COX-2 and renin gene expression (40). Macula densa COX-2 expression is known to be augmented by dietary salt restriction and renal artery constriction, two conditions in which renin gene expression is also increased (17, 18, 42, 46). A functional connection is suggested by the finding that COX-2 inhibition prevented the increase in renal renin content and renin mRNA caused by dietary Na restriction (16). Ureteral obstruction and diuretic-induced loop of Henle transport inhibition are two other conditions where a chronic reduction of NaCl transport by the macula densa may be associated with increased COX-2 and renin expression (40). A local rather than systemic mechanism for control of COX-2 expression is supported by observations that renal medullary COX-2 is typically not stimulated by the same interventions. In fact, a dietary Na restriction causes a decrease rather than an increase in inner medullary COX-2 (23, 46).

In conclusion, the results of the present studies in the isolated perfused TAL/glomerulus preparation of the rabbit show that COX-2 inhibition by NS-398 completely prevented the stimulation of renin secretion caused by a decrease in luminal NaCl concentration. In contrast, NaCl-dependent stimulation of renin secretion was unaffected by COX-1 inhibition. These results suggest that COX-2 in epithelial cells in the tubulovascular contact region is a critical component of macula densa control of renin secretion, presumably through release of the renin-stimulating prostaglandins PGE$_2$ and PGF$_2\alpha$.

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-37448, DK-39255, and DK-40042.

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Received 23 March 1999; accepted in final form 11 June 1999.

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