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tubule-to-granular cell signaling pathway that regulates the release of renin.

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, MI) 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 nGUs/min. During perfusion with the low-NaCl solution (period 2), renin secretion increased to a mean value of 26.1 ± 7.4 nGUs/min (P < 0.01, n = 19). Return to the high-NaCl solution (period 3) caused average renin secretion to fall to 10.4 ± 4 nGUs/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 nGUs/min in the initial high-NaCl period, 30.5 ± 9.4 nGUs/min during the low-NaCl perfusion period (P < 0.01, n = 8), and 11.6 ± 3.7 nGUs/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).](image-url)
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 isofoms 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 isofom typically induced by inflammatory citoikines, is constiu tively 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 isofoms, and by implication which cell type, is involved in macula densa control of renin secretion by using isofom-specific inhibitors of COX activity. Our data show that NS-398, a COX-2-specific inhibitor, prevented NaCl-dependent changes in renin secretion, whereas valersalicylate, a COX-1 inhibitor, had no effect. NS-398 has been shown to inhibit the activity of purified COX-2 with an IC50 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, valersalicylate has a narrower window of selectivity, with the IC50 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 A2 (cPLA2), which is activated by calcium in the 10−7 M concentration range (14, 32). Thus, if a reduction in luminal NaCl causes cytosolic Ca2+ to increase, then cPLA2 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

![Graph showing mean renin secretion rates during perfusion with high- and low-NaCl solutions in control specimen (left) and in presence of valersalicylate (middle) or NS-398 (right). *P < 0.01 comparing low-NaCl vs. initial high-NaCl period.](http://ajprenal.physiology.org/)
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 activity of neuronal nitric oxide synthase (nNOS) may be a determinant of COX-2 activity in the same cells. Our previous observation in the same 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 PGE2 and/or PGI2 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 PGE2 receptors of the EP4 and possibly EP2 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). PGE2 receptors of the EP2/EP3 subtype are Gi coupled and cause an activation of adenylate cyclase (6). It is consistent with this signal transduction mechanism that PGE2 and PGI2 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 PGE2 and PGI2.

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REFERENCES


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NS-398 INHIBITS MACULA Densa-MEDIATED RENIN SECRERTION


