Role of 20-HETE in Elevating Chloride Transport in the Thick Ascending Limb of Dahl SS/Jr Rats

Osamu Ito, Richard J. Roman

Abstract—This study examined the role of endogenous 20 hydroxyeicosatetraenoic acid (20-HETE) in elevating Cl− transport in the medullary thick ascending loop of Henle (MTAL) of 9-week-old male Dahl salt-sensitive (SS/Jr) and salt-resistant (SR/Jr) rats perfused in vitro. Basal transepithelial voltage (V_{te}; 14.9±0.9 versus 10.1±0.5 mV) and net lumen-to-bath Cl− flux (J_{Cl}); (155±6 versus 127±5 pEq·min⁻¹·mm⁻¹) were significantly greater in MTAL isolated from SS/Jr rats (n=16) than in those obtained from SR/Jr rats (n=16). Blockade of the synthesis of 20-HETE with 17-octadecynoic acid (17-ODYA; 10 μmol/L) increased V_{te} from 9.9±0.8 to 13.1±1.0 mV and J_{Cl} from 127±7 to 152±8 pEq·min⁻¹·mm⁻¹ in the MTAL of SR/Jr rats (n=8), but it had no significant effect on V_{te} or J_{Cl} in the MTAL of SS/Jr rats (n=8). Exogenous 20-HETE (1 μmol/L) decreased V_{te} from 14.8±0.6 to 10.5±0.6 mV and J_{Cl} from 155±10 to 116±6 pEq·min⁻¹·mm⁻¹ in MTAL of SS/Jr rats (n=8), but it had no effect on V_{te} or J_{Cl} in the MTAL of SR/Jr rats (n=8). The expression of P4504A2 protein in the MTAL of SS/Jr rats was approximately half of that seen in the MTAL of SR/Jr rats. These results indicate that endogenously formed 20-HETE regulates transepithelial voltage and Cl− transport in the MTAL and that a diminished production of 20-HETE contributes to an elevation in Cl− transport in the MTAL of SS/Jr rats. (Hypertension. 1999;33(part II):419-423.)

Key Words: kidney ■ sodium ■ potassium ■ ion transport ■ cytochrome P450 ■ hypertension, salt-sensitive ■ blood pressure

Renal transplantation studies have indicated that some form of renal dysfunction underlies the development of hypertension in Dahl salt-sensitive (SS/Jr) rats; however, the factors responsible for altering kidney function are still unknown. We and others have reported that the pressure-natriuresis relationship is reset toward higher pressures before the development of hypertension in Dahl salt-sensitive/John Rapp substrain (SS/Jr) rats. Previous micropuncture studies have indicated that Cl− reabsorption is elevated in the loop of Henle, somewhere between the accessible portions of the late proximal and early distal tubule in Dahl SS/Jr rats. However, the exact nephron segment involved could not be identified because this portion of the nephron spans 4 distinct segments, including the Pars recta, thin ascending and descending limbs, and the thick ascending loop of Henle. Moreover, it remains to be determined whether the alterations in sodium and chloride reabsorption seen in Dahl SS/Jr rats are related to intrinsic differences in the regulation of ion transport in a specific cell type in the kidney or are secondary to strain differences in renal hemodynamics, physical factors, the levels of circulating hormones and paracrine factors, or all of these that modify sodium transport in vivo.

Recent observations have suggested that changes in the renal metabolism of arachidonic acid by enzymes of the P4504A family may contribute to the elevation in loop Cl− transport and the development of hypertension in Dahl SS/Jr rats. In this regard, the formation of 20 hydroxyeicosatetraenoic acid (20-HETE) is reduced, and the levels of P4504A protein are lower in the renal outer medulla of SS/Jr rats than in normotensive strains of rats. Previous studies have also indicated that 20-HETE is the primary metabolite of arachidonic acid that is produced in the thick ascending limb of the loop of Henle. 20-HETE is a potent inhibitor of Na+–K⁺–2Cl− transport in these cells. Thus, it is possible that a deficiency in the formation of this substance could contribute to the elevation in loop Cl− transport seen in SS/Jr rats. Our recent finding that in vivo perfusion of the loop of Henle of SS/Jr rats with exogenous 20-HETE normalizes loop Cl− transport supports this view. This hypothesis is also consistent with previous observations that (1) a genetic marker within P4504A2 gene cosegregates with blood pressure in a F2 cross of SS/Jr and Lewis rats, (2) induction of the renal production of 20-HETE attenuates the development of hypertension in SS/Jr rats, and (3) chronic inhibition of 20-HETE formation in the outer medulla induces salt-sensitive hypertension in Lewis rats.

The purpose of the present study was to determine whether Cl− transport and transepithelial potential are elevated in the medullary thick ascending limb of Henle (MTAL) of SS/Jr rats perfused in vitro and to compare the effects of blockade

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of 20-HETE formation with 17-octadecynoic acid (17-ODYA) and exogenous 20-HETE on Cl⁻ transport in the MTAL of SS/Jr and SR/Jr rats. In addition, the expression of P4504A protein was compared in the MTAL of Dahl SS/Jr rats and of a salt-resistant/John Rapp strain (SR/Jr) of rats.

**Methods**

Experiments were performed on 9-week-old male SS/Jr, SR/Jr, Lewis, and Sprague-Dawley rats. The SR/Jr, Lewis, and Sprague-Dawley rats were purchased from Harlan Laboratories (Indianapolis, IN), and the SS/Jr rats were obtained from a colony maintained at the Medical College of Wisconsin by brother-sister mating since 1991. The rats were housed in an Animal Care Facility that is approved by the American Association for Accreditation of Laboratory Animal Care. The rats were maintained on a low salt diet (0.4% NaCl) throughout the study. All protocols received prior approval by the Animal Welfare Committee of the Medical College of Wisconsin.

**In Vitro Microperfusion of the MTAL**

The rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (30 mg/kg). MTALs were dissected from the outer medulla with fine forceps and perfused in vitro at a rate of 20 nL/min, according to the technique of Burg et al. Bath temperature was maintained at 37°C, and the perfusion and the bath solutions contained (in mmol/L) 135 NaCl, 3 KCl, 1.5 CaCl₂, 1 MgCl₂, 2 KH₂PO₄, 5.5 glucose, 5 L-alanine, and 10 HEPES (pH 7.4) and were equilibrated with 100% oxygen.

Transmepithelial voltage (Vₑ) was measured between Ag-AgCl wires inserted into 0.9% NaCl-4% agar bridges that were attached to the perfusion pipette and the bath. Net lumen-to-bath Cl⁻ flux (Jₑ) was also measured in some experiments. In these experiments, the perfusion rate was lowered to 5 nL/min, and the Cl⁻ concentration of the perfuse and collected fluid was measured with a microtitrator (model F-25; WP).

Control Vₑ and Jₑ were measured after a 60-minute equilibration period. Then, 17-ODYA (10 μmol/L), an inhibitor of the formation of 20-HETE, or 20-HETE (1 μmol/L) was added to the bath, and 20 minutes later Vₑ and Jₑ were re determined.

**Bulk Isolation of MTAL**

MTALs were isolated with the method of Trinh-Trang-Tan et al. Densitometer SI; Molecular Dynamics).

**Immunoblot Analysis**

Proteins were separated by electrophoresis on a 10x359

**Results**

**Basal Cl⁻ Transport in the MTAL of Dahl SS/Jr and Normotensive Rats**

A comparison of Vₑ and Jₑ in MTAL of SS/Jr and SR/Jr rats is presented in Figure 1. Basal Vₑ in the MTAL of Dahl SS/Jr rats (n=16) was significantly greater than that seen in SR/Jr rats (n=16) or in normotensive Sprague-Dawley (10.2±1.2 mV, n=8) or Lewis rats (6.6±0.9 mV, n=8). Baseline Jₑ in the MTAL of SS/Jr rats was also significantly greater than that seen in SR/Jr rats (Figure 1).

**Effect of 17-ODYA on Cl⁻ Transport in the MTAL**

A comparison of the effects of blocker of 20-HETE formation with 17-ODYA on Vₑ and Jₑ in the MTAL of SS/Jr and SR/Jr rats is presented in Figure 2. The addition of 17-ODYA (10 μmol/L) to the bath had no effect on Vₑ and Jₑ in the MTAL of SS/Jr rats. In contrast, Vₑ and Jₑ increased significantly in the MTAL of SR/Jr rats after the addition of 17-ODYA. A similar effect of 17-ODYA on Vₑ was also observed in the MTAL of normotensive Sprague-Dawley rats (from 10.2±1.2 to 13.8±1.3 mV, n=8, P<0.001) and Lewis rats (from 6.6±0.9 to 8.6±0.9 mV, n=8, P<0.005). To determine whether this effect was specific to blockade of the metabolism of arachidonic acid by P-450, we examined the effects of indomethacin on Vₑ in the MTAL of SR/Jr rats. The addition of indomethacin (10 μmol/L) to the bath had no significant effect on Vₑ (10.3±0.7 versus 10.3±0.6 mV, n=8) in these experiments.

**Effect of 20-HETE on Cl⁻ Transport in the MTAL**

A comparison of the effects of exogenous 20-HETE (1 μmol/L) on Vₑ and Jₑ in the MTAL of SS/Jr and SR/Jr rats
is presented in Figure 3. The addition of 20-HETE (1 μmol/L) to the bath significantly reduced \( V_t \) and \( J_{Cl} \) in the MTAL of SS/Jr rats, but it had no significant effect in the MTAL of SR/Jr rats.

Expression of P4504A Proteins in the MTAL of Dahl Rats

A comparison of the levels of P4504A enzymes responsible for the formation of 20-HETE in the MTAL of SS/Jr and SR/Jr rats is presented in Figure 4. One immunoreactive band was detected in homogenates prepared from the MTAL of SS/Jr and SR/Jr rats. In comparison with rat liver standards, this isoform migrated like the P4504A2 isoform. The level of P4504A protein in the MTAL of SR/Jr rats was twice as high as that seen in the MTAL of SS/Jr rats. In other experiments, we confirmed that the levels of P4504A protein were also higher in MTAL isolated from Sprague-Dawley and Lewis rats than in MTAL of Dahl SS/Jr rats (data not shown).

Discussion

The present study examined whether \( Cl^- \) transport is elevated in the MTAL of Dahl SS/Jr perfused in vitro and the hypothesis that a deficiency in the production of 20-HETE in the MTAL contributes to alterations in the renal handling of sodium and chloride previously reported in this strain of rats in vivo.\(^2\)\(^-\)\(^6\) The results indicate that \( Cl^- \) reabsorption is elevated in the MTAL of SS/Jr rats perfused in vitro as compared with that seen in the MTAL of SR/Jr rats. \( V_{sc} \) is also 30% higher in the MTAL of SS/Jr rats as compared with the levels seen in normotensive SR/Jr, Sprague-Dawley, and Lewis strains of rats. The significance of these findings is that they are the first to identify the MTAL as the renal cell type in which tubular reabsorption of sodium and chloride is elevated in SS/Jr rats. Indeed, all previous work has indicated that sodium reabsorption is actually reduced in all other nephron segments in Dahl SS/Jr rats, perhaps as an adaptation to chronic sodium retention.\(^7\)

Another important aspect that we found is that there is a phenotypic difference in the regulation of \( Cl^- \) transport in the MTAL of Dahl SS/Jr rats that can still be demonstrated in vitro and that is therefore independent of strain differences in renal hemodynamics, the levels of circulating hormones and paracrine factors, or both that influence sodium reabsorption in vivo. Thus, measurement of \( V_{sc} \) and \( J_{Cl} \) in the MTAL may serve as valuable intermediate phenotypes to identify chro-
mosomal regions and candidate genes that influence salt-sensitivity in this model. Moreover, the present findings suggest that all of the factors that regulate Cl⁻ transport in the MTAL can now be considered to be viable candidate genes for the development of hypertension. These include subunits of Na⁺,K⁺-ATPase, the Na⁺,K⁺,2Cl⁻ cotransporter, and the 70- and 30-pS apical K⁺ channels that regulate Vₑ in the MTAL. Indeed, a molecular variant of the α subunit of Na,K-ATPase has been linked to alterations in blood pressure and red cell transport in Dahl SS/Jr rats. Moreover, mutations in the Na⁺,K⁺,2Cl⁻ cotransporter have also been shown to alter blood pressure in humans. It should also be noted that the activity of this transporter is regulated by hormones and paracrine factors that act via the cAMP, cGMP, protein kinase C, and cytochrome P-450/arachidonic acid pathways. Thus, components of these signal transduction pathways can also be considered as potential candidate genes for salt-sensitivity in Dahl SS/Jr rats.

Previous studies have indicated that 20-HETE is the primary metabolite of arachidonic acid produced in thick ascending limb cells of rabbits and that 20-HETE inhibits rubidium uptake, an indirect index of Na⁺,K⁺,2Cl⁻ cotransport, in these cells. Moreover, recent patch-clamp studies have indicated that 20-HETE blocks an apical K⁺ channel in the MTAL. Blockade of this channel should reduce Vₑ and the availability of K⁺ for reuptake by the Na⁺,K⁺,2Cl⁻ cotransporter in the MTAL. These findings have led to the suggestion that 20-HETE plays a key role in the regulation of sodium and Cl⁻ transport in this segment of the nephron. However, this hypothesis has yet to be directly tested in an intact tubule. The present findings that blockade of the formation of 20-HETE with 17-ODYA increased Vₑ in the MTAL of 3 different strains of rats now provides the first direct evidence that endogenously formed cytochrome P-450 metabolites regulate Cl⁻ transport and Vₑ in this nephron segment. These findings also indicate that 20-HETE probably limits passive reabsorption of Na⁺, Ca²⁺, and Mg²⁺ in the MTAL because the transepithelial potential provides the major driving force for the passive movement of cations in this segment. The results of the present study are also consistent with recent studies indicating that the inhibitory effects of Ca²⁺, bradykinin, tumor necrosis factor, endothelin, and angiotensin II on sodium transport in the thick ascending limb are attenuated by cytochrome P-450 inhibitors and likely are mediated by 20-HETE.

The present study also examined the hypothesis that a deficiency in the production of 20-HETE contributes to the elevation in Cl⁻ transport and Vₑ in the MTAL of Dahl SS/Jr rats. The results indicate that the expression of the P4504A2 enzyme responsible for the formation of 20-HETE is reduced in the MTAL of SS/Jr rats relative to the levels seen in the MTAL of SR/Jr rats. Similar results were seen when the levels of P4504A protein in the MTAL of SS/Jr were compared with those seen in Sprague-Dawley or Lewis rats. This finding is consistent with our previous studies indicating that the production of 20-HETE is reduced in microsomes prepared from the outer medulla of Dahl SS/Jr rats relative to levels seen in SR/Jr and Lewis rats. Moreover, our in vitro microperfusion studies now provide direct functional evidence that a deficiency in the production of 20-HETE contributes to the elevation in Cl⁻ transport and Vₑ in the MTAL of Dahl SS/Jr rats. In this regard, we found that an inhibitor of the production of 20-HETE enhanced Vₑ and Cl⁻ transport in the MTAL of SR/Jr, Sprague-Dawley, and Lewis rats to the same level as that seen in the MTAL of SS/Jr rats. However, it had no effect on Cl⁻ transport in the MTAL of SS/Jr rats in which the endogenous levels of 20-HETE were perhaps below the threshold concentration needed to inhibit Cl⁻ transport. Moreover, exogenous 20-HETE reduced Cl⁻ transport in the MTAL of SS/Jr rats, but it had no effect on Cl⁻ transport in Dahl SR/Jr rats in which the endogenous levels of 20-HETE might already have been high enough to maximally reduce baseline Vₑ.

In summary, the results of the present study indicate that there is a phenotypic elevation in Cl⁻ transport in the MTAL of Dahl SS/Jr rats and provides direct evidence that there is differential expression of a gene (P4504A) that cosegregates with blood pressure in the renal cell type (MTAL) in which sodium transport is elevated in Dahl SS/Jr rats. The results also indicate that 20-HETE produced by the P4504A enzyme exerts a tonic inhibitory influence on Cl⁻ transport in the MTAL and provides functional evidence that a deficiency in the formation of 20-HETE contributes to the elevation in Cl⁻ transport in the MTAL of Dahl SS/Jr rats. Overall, these findings provide a strong rationale for further evaluation of the P4504A gene as a candidate for mediating salt-sensitivity in Dahl SS/Jr rats.

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References


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