Role of Na\(^+/\)H\(^+\) exchanger NHE3 in nephron function: micropuncture studies with S3226, an inhibitor of NHE3

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Vallon, Volker, Jan-Robert Schwark, Kerstin Richter, and Max Hropot. Role of Na\(^+/\)H\(^+\) exchanger NHE3 in nephron function: micropuncture studies with S3226, an inhibitor of NHE3. Am. J. Physiol. Renal Physiol. 278: F375–F379, 2000.—Na\(^+/\)H\(^+\) exchanger NHE3 is expressed in the luminal membrane of proximal tubule and thin and thick ascending limb of Henle’s loop. To further define its role, the novel NHE3 inhibitor S3226 was employed in micropuncture experiments in nephrons with superficial glomeruli of anesthetized rats. Micropuncture of proximal convoluted tubule with S3226 revealed a dose-dependent inhibition of reabsorption (IC\(_{50}\) of 4–5 µM) with a maximum inhibition of 30% for fluid and Na\(^+\). During micropuncture of Henle’s loop (last superficial proximal to first superficial distal tubular loop), no effect of S3226 (10 or 30 µM) on the reabsorption of fluid or Na\(^+\) was observed. Finally, S3226 (30 µM) left the tubuloglomerular feedback response unaltered as determined by the fall in proximal tubular stop-flow pressure in response to increasing loop of Henle perfusion rate. These studies indicate that NHE3 significantly contributes to fluid and Na\(^+\) reabsorption in proximal convoluted tubule. NHE3 appears not to significantly contribute to fluid or Na\(^+\) reabsorption in the loop of Henle (including the S3 segment of proximal tubule) or macula densa control of nephron filtration.

reabsorption; proximal tubule; loop of Henle; tubuloglomerular feedback

A significant portion of proximal tubule sodium ion reabsorption is mediated by apical membrane Na\(^+/\)H\(^+\) exchanger (12, 13). Thus far, five Na\(^+/\)H\(^+\) exchanger (NHE) isoforms have been cloned (3, 10, 14, 15, 17, 18), and Northern blotting analysis has detected the mRNA of four of the NHE isoforms (NHE1–4) in the mammalian kidney (10, 15, 17, 18). Immunocytochemical studies indicate that NHE3 is the principal Na\(^+/\)H\(^+\) exchanger isoform expressed on proximal tubular brush-border membrane, suggesting a substantial role of NHE3 in sodium ion and fluid reabsorption in proximal tubule (1, 2, 5, 7).

Whereas cariporide (HOE-642) is known selective inhibitor of NHE1 (19, 20), a comparably selective inhibitor for one of the other NHE isoforms had not been available. More recently, however, Schwark and co-workers (20) showed that 3-[2-[(3-guanidino-2-methyl-3-oxo-propenyl)-5-methyl-phenyl]-N-isopropylidene-2-methyl-acrylamide dihydrochloride (S3226) is a selective inhibitor of NHE3. In the latter study, selectivity for NHE3 was demonstrated in a mouse fibroblast L cell line (LAP1) transfected with human NHE1, rabbit NHE2, rat or human NHE3, as well as an opossum kidney cell line and porcine renal brush-border membrane vesicles (20).

The first purpose of the present study was thus to assess the effect of intratubular application of the NHE3 inhibitor S3226 on reabsorption of Na\(^+\) and fluid in proximal tubule in the anesthetized rat. To get relatively close to the very early proximal tubule, studies were performed in nephrons with superficial glomeruli of Munich-Wistar-Frömter rats. Because it had been observed in vitro that higher concentrations of S3226 can inhibit NHE1 (20), additional experiments on proximal convoluted tubule reabsorption were performed with the NHE1 inhibitor HOE-642 (19, 20). Whereas there appears no doubt as to the expression of NHE3 in both the S1 and S2 segment of the proximal tubule, conflicting evidence was published with regard to the presence of NHE3 in the S3 portion of rat proximal tubule: Amemiya and co-workers (2) could detect NHE3 only in S1 and S2 segments of proximal tubule. In comparison, Bliemelseder and co-workers (7) reported staining for NHE3 in the entire proximal tubule including the S3 segment. Besides the proximal tubule, there is evidence that NHE3 is expressed in the apical membrane of thin and thick limbs of the loop of Henle (1, 2, 7). Thus the second purpose of the study was to assess whether NHE3 contributes to Na\(^+\) and fluid reabsorption in the loop of Henle (including the S3 portion of the proximal tubule). Finally, we assessed the effect of intratubular S3226 on tubuloglomerular feedback (TGF) because a possible role of Na\(^+/\)H\(^+\) exchange in macula densa signaling has recently been proposed by Bell and co-workers (4, 9, 11).

METHODS

Studies were conducted in adult male Munich-Wistar-Frömter rats weighing 270 to 350 g. The animals were allowed free access to tap water and a regular rat pellet diet (0.25% Na\(^-\); estimated Na\(^+\) excretion based on previous studies in metabolic cages with same diet of ~2 mmol/24 h (22)). The preparation for micropuncture was carried out according to standard protocols previously described (21, 23, 24). Briefly, animals were anesthetized with thiobutabarbital (120 mg/kg body wt ip; Research Biochemicals International), and body temperature was maintained at 37°C. After trache-
ostomy (PE-200) and placement of catheters (PE-50) in jugular vein, femoral artery, and urinary bladder, the left kidney was exposed by flank incision, immobilized in a Lucite cup with warmed agar (3%), and covered with warm paraffin oil, and the left ureter was cannulated (PE-50). Studies were performed under infusion of Ringer saline (in mM: 30 NaCl, 4.7 KCl, and 111 NaClO 3 at 1.5 ml/h plus 0.85% NaCl at 1.5 ml/min. Arterial blood pressure was monitored continuously (P23dB Gould-Statham pressure transducer, Oxnard, CA) throughout each experiment. The rats were allowed 90 min to stabilize before micropuncture experiments were started.

The following types of artificial tubular perfusion fluids (ATF) were employed. Type I ATF applied to the early proximal tubule comprised (in mM) 113 NaCl, 25 NaHCO 3, 4 KCl, 1 MgSO 4, 2 CaCl 2, 1 Na 2HPO 4, 5 glucose, and 5 urea, 0.075% FD&C green, pH 7.4. Type II ATF applied to the late proximal tubule comprised (in mM) 130 NaCl, 10 NaHCO 3, 4 KCl, 2 CaCl 2, and 7.5 urea, 0.075% FD&C green, pH 7.4.

Series 1: Effect of intratubular S3226 on reabsorption in proximal convoluted tubule or loop of Henle. Tubular reabsorption was studied during microperfusion of functionally isolated 1) proximal convoluted tubule or 2) loop of Henle as previously described (24) with or without S3226 added to the perfusate. In experiments on proximal convoluted tubule, HOE-642 was also tested. Briefly, a perfusion pipette directly attached to a calibrated Hampel microperfusion pump and filled with the appropriate type of ATF was inserted into 1) the first surface loop of the proximal convoluted tubule or 2) the first accessible distal tubular loop, and a timed collection of tubular fluid was obtained. In the last surface loop, the last accessible loop of tubular fluid was obtained. Subsequently, a second perfusion pipette connected to a second Hampel pump (pump 2) and filled with ATF (vehicle) or ATF containing S3226 or HOE-642 was inserted in close proximity to the first pump. Pump 1 was switched off, and pump 2 was started. After a time period of 5 min, a second tubular collection was performed.

Series 2: Effect of intratubular S3226 on TGF. To assess the effect of S3226 on TGF, the stop-flow-pressure (SFP) response to changes in late proximal perfusion rate (Vp) was determined during orthograde perfusion of Henle's loop as previously described (24) with or without S3226 added to the perfusate. After identification of nephron configuration, a perfusion pipette directly attached to a calibrated Hampel microperfusion pump was inserted into the first surface loop of the proximal tubule. Two immobile wax blocks were injected into the proximal tubule, one upstream from the perfusion pipette inserted into the last accessible loop and the other into the first accessible loop of the proximal tubule. Another micropipette (1- to 3-µm tip) filled with NaCl (1.5 M) and connected with a servo-nulling device (WPI, New Haven, CT) was inserted upstream from the proximal wax block to monitor early proximal SFP during late proximal perfusion with type II ATF at rates of 10, 15, 20, 25, 30, and 40 nl/min to obtain basal measurements. Subsequently, a second perfusion pipette connected to a second Hampel pump (pump 2) and filled with ATF (vehicle) or ATF containing S3226 was inserted in close proximity to the first pump. Pump 1 was switched off, and pump 2 was started to reassess the SFP response during loop of Henle perfusion at 10, 15, 20, 25, 30, and 40 nl/min.

Materials and analytic methods. Tubular flow rate was measured by utilizing a constant bore capillary. Na + and K + concentration in the collected tubular fluid were determined as previously described (23, 24) employing a micro flame photometer, which was developed and built by Rolf Englert and Klaus Stieler (Dept. of Pharmacology, Univ. of Tübingen) on the basis of the original conception by Wolfgang Hampel (Wissenschaftlicher Gerätebau, Frankfurt/Main, Germany). Tubular fluid samples from late proximal tubule, Cl + concentration was measured with a microadaptation of the electrometric titration method as previously described (23). The equipment for the latter (Microtitre ET-1, World Precision Instruments, Sarasota, FL) was kindly placed at our disposal by Prof. D. Häsler (Dept. of Physiology, Univ. of Munich).

Statistical methods. A paired t-test was performed to analyze data in series 1 and 2. P < 0.05 is considered to be statistically significant.

RESULTS

Series 1A: Effect of intratubular S3226 on reabsorption in proximal convoluted tubule. Basal control measurements for fractional reabsorption of fluid, Na +, and Cl + in the perfused proximal tubule segments were not different among groups of nephrons subsequently perfused with either vehicle, the various concentrations of S3226, or HOE-642 and averaged 35, 42, and 45%, respectively (data not shown). Whereas time control experiments with vehicle revealed no significant change, S3226 was found to elicit a dose-dependent inhibition of fluid, Na +, and Cl + reabsorption in proximal convoluted tubule with an IC 50 of 4–5 µM (Fig. 1). Maximum inhibition by S3226 of fluid and Na + reabsorption were both ~30%. Maximum inhibition of Cl + reabsorption was ~10%. The fractional change elicited by S3226 at 10 or 30 µM in Na + reabsorption was significantly greater than the change in Cl + reabsorption (P < 0.05 for each paired comparison). Intratubular application of HOE-642 (10 µM) did not elicit a significant change in fluid, Na +, or Cl + reabsorption in proximal convoluted tubule (−3.8 ± 4.3, −3.5 ± 3.4, or −1.8 ± 2.4%, respectively, n = 8, not significant compared with basal control measurements).

Series 1B: Effect of intratubular S3226 on reabsorption in the loop of Henle. During microperfusion of Henle's loop, no effect of intratubular S3226 (10 or 30 µM) on the reabsorption of fluid, Na +, or K + was observed (Fig. 2).

Series 2: Effect of intratubular S3226 on TGF. Application of S3226 (30 µM) neither changed the maximum response in SFP nor apparently shifted the SFP response curve or its slope around the operating point (Fig. 3).

DISCUSSION

In the present micropuncture studies we employed S3226, a recently identified potent inhibitor of NHE3...
(20), to further elucidate the role of NHE3 in nephron function under in vivo conditions in the rat. It was first observed that intratubular application of S3226 elicited a dose-dependent inhibition of fluid and Na\(^{+}\) reabsorption in proximal convoluted tubule, with an IC\(_{50}\) of 4–5 µM and a maximum inhibition of \(\approx 30\%\). Potential effects of S3226 on NHE1 in the latter response were ruled out by demonstrating that the selective NHE1 inhibitor HOE-642 did not significantly alter the reabsorption of fluid or Na\(^{+}\) in proximal convoluted tubule.

These findings indicated a significant role of NHE3 in proximal tubule Na\(^{+}\) and fluid reabsorption in vivo and are in accordance with the prominent expression of NHE3 in proximal tubule brush border (1, 2, 5, 7). Consistent with inhibition of Na\(^{+}/H^{+}\) exchange in proximal convoluted tubule by S3226, it was further observed that the fall in fluid and Na\(^{+}\) reabsorption was significantly more pronounced than the change in Cl\(^{-}\) reabsorption, which fell by a maximum of \(\approx 10\%\). Inhibition of Na\(^{+}/H^{+}\) exchange by S3226 may have partly lowered the transport of Cl\(^{-}\) in proximal convoluted tubule occurring through paracellular solvent drag. At the same time, however, the inhibition of H\(^{+}\) recycling to the lumen may have lowered HCO\(_{3}^{-}\) reabsorption and thus increased luminal concentrations of HCO\(_{3}^{-}\). The latter is expected to facilitate Cl\(^{-}\) reabsorption. Basically consistent with the present study, it was reported recently by Schultheis and co-workers (16).

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Fig. 1. Effect of intratubular S3226 on reabsorption in proximal convoluted tubule. After control measurement with perfusion of artificial tubular fluid (ATF) only, recollection was performed during application of experimental perfusate [ATF (vehicle) or ATF + S3226]. Circles plus error bars indicate mean values ± SE; \(n = 5–9\) nephrons/group. Notice that S3226 elicited a dose-dependent inhibition of fluid, Na\(^{+}\), and Cl\(^{-}\) reabsorption in proximal convoluted tubule with an IC\(_{50}\) of 4–5 µM. Maximum inhibition by S3226 of fluid and Na\(^{+}\) reabsorption were \(\approx 30\%\). Maximum inhibition of Cl\(^{-}\) reabsorption by S3226 was \(\approx -10\%\). * \(P < 0.05\) vs. control measurement.

Fig. 2. Effect of intratubular S3226 on fractional Na\(^{+}\) reabsorption in loop of Henle. After control measurement with perfusion of artificial tubular fluid (ATF) only (CON), recollection was performed during application of experimental perfusate [ATF (vehicle) or ATF + S3226]. Circles plus error bars indicate mean values ± SE; \(n = 5–7\) nephrons/group. Notice that S3226 did not significantly alter fractional reabsorption of fluid, Na\(^{+}\), or K\(^{+}\) in Henle's loop (FR-fluid (A), FR-Na\(^{+}\) (B), FR-K\(^{+}\) (C), respectively).

Fig. 3. Effect of intratubular S3226 on tubuloglomerular feedback. Depicted is stop-flow-pressure (SFP) response curve during gradually increasing loop of Henle perfusion rate. Circles plus error bars indicate mean values ± SE. Paired measurements were performed in 6 nephrons before (vehicle) and during addition of S3226 to ATF. Notice that application of S3226 neither changed maximum response in SFP nor apparently shifted curve or affected its slope.
that, in a mouse model in which the gene encoding NHE3 has been ablated using gene-targeting strategies, both fluid and HCO_3^- reabsorption were significantly reduced during in vitro perfusion of proximal tubule segments.

Whereas expression of NHE3 in the brush border of both the S1 and S2 segment of the proximal tubule was consistently reported (1, 2, 5, 7), conflicting evidence was published with regard to the presence of NHE3 in the S3 portion of rat proximal tubule. Amemiya and co-workers (2) could detect NHE3 in S1 and S2 but not the S3 segment of proximal tubule. These authors pointed out that their inability to detect NHE3 in the S3 segment is consistent with decreased HCO_3^- reabsorption in the straight proximal tubule, which in turn may reflect a lower amount of NHE3 expression. In comparison, Biemesderfer and co-workers (7) reported staining for NHE3 in the brush border of the entire proximal tubule including the S3 segment. Besides the proximal tubule, there is evidence that NHE3 is expressed in the apical membrane of thin and thick limbs of the loop of Henle (1, 2, 7). In the present study it was observed that intratubular S3226 at concentrations of 10 or 30 µM did not significantly alter the reabsorption of fluid, Na^+, or K^+ in the segment between the last superficial proximal tubular loop and the first accessible distal tubular loop. Whereas NHE3 may play a role in cellular and tubular fluid acidification in Henle’s loop, the above findings indicate that NHE3 did not significantly contribute to the net reabsorption of fluid, Na^+, or K^+ in the S3 segment of proximal tubule, or the thin or thick ascending limb of Henle’s loop.

A possible role of Na^+/H^+ exchange in macula densa signaling has recently been proposed by Bell and co-workers (4, 9, 11), who have reported apical and basolateral Na^+/H^+ exchange activity in macula densa cells. Furthermore, immunohistochemical studies provided evidence that both NHE3 and NHE2 are expressed in the apical membrane of cortical thick ascending limb up to and probably including the macula densa (2, 8). In addition, NHE1 appears to be ubiquitously expressed in the basolateral membrane of most nephron segments (6). In the present study it was observed that intratubular S3226 at a concentration of 30 µM did not significantly alter the TGF response, which was assessed as the fall in early proximal tubular SFP in response to an increasing loop of Henle perfusion rate. Because S3226 did not apparently alter the reabsorption of fluid, Na^+, or K^+ in Henle’s loop and therefore most likely left the luminal signal of the TGF unaltered during loop perfusion, these findings indicated that NHE3 did not significantly contribute to the reading of the luminal TGF signal or signal transduction by macula densa cells.

In summary, the present studies in superficial nephrons of the anesthetized rat indicate that NHE3 significantly contributes to fluid and Na^+ reabsorption in proximal convoluted tubule. In comparison, NHE3 appears not to significantly contribute to fluid or Na^+ reabsorption in the loop of Henle (including the S3 segment of proximal tubule) or macula densa control of nephron filtration.

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