Na\(^+\)/H\(^+\)-exchange activity and immunolocalization of NHE3 in rat epididymis

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Bagnis, Corinne, Mireille Marsolais, Daniel Biemesderfer, Raynard Laprade, and Sylvie Breton. Na\(^+\)/H\(^+\)-exchange activity and immunolocalization of NHE3 in rat epididymis. Am J Physiol Renal Physiol 280: F426–F436, 2001.—An acidic luminal pH in the epididymis and vas deferens (VD) helps maintain mature sperm in an immotile state during storage. We have previously shown that the majority of proton secretion in the VD is due to the activity of the vacuolar H\(^+\)-ATPase. Acidification is dependent on luminal sodium in more proximal regions of the epididymis, and we examined the distribution of the Na\(^+\)/H\(^+\) exchanger, NHE3, by immunofluorescence and measured Na\(^+\)/H\(^+\) exchange (NHE) activity in isolated epididymal tubules. NHE3 was detected in the apical pole of nonciliated cells of the efferent ducts and principal cells (PC) of the epididymis. No staining was seen in the distal cauda epididymis and the VD. Isolated tubules from the distal initial segment (DIS) and proximal cauda epididymis were perfused in vitro and loaded with the pH-sensitive dye 2′,7′-bis(carboxyethyl)-5(6′)-carboxyfluorescein. Ethylisopropyl amiloride (EIPA) (50 \(\mu\)M) reduced the initial rate of intracellular pH recovery (dpH/dt), in response to an acute acid load, by 51% and 45% in the DIS and cauda epididymis, respectively. In the DIS, removal of luminal sodium reduced dpH/dt by 52%. HOE694 (50 \(\mu\)M) inhibited all EIPA-sensitive dpH/dt in the DIS, despite the previously reported absence of NHE2 in this region (Cheng Chew SB, Leung GPH, Leung PY, Tse CM, and Wong PYD, Biol Reprod 62: 755–758, 2000). These data indicate that HOE694- and EIPA-sensitive Na\(^+\)/H\(^+\) exchange may participate, together with the H\(^+\)-ATPase, in luminal acidification in the male efferent duct.

Biology of the epididymis and vas deferens: hormonal and environmental factors affecting sperm maturation.

Male reproductive tract; transepithelial acid-base transport; acidification; immunofluorescence; intracellular pH.

MEMBERS OF THE SODIUM/HYDROGEN exchanger (NHE) family mediate, under physiological conditions, the electroneutral exchange of one extracellular Na\(^+\) for one intracellular H\(^+\) across the plasma membrane. In eukaryotic organisms, the NHEs are involved in a wide variety of physiological processes including intracellular pH (pHi) regulation, cell volume regulation, as well as transepithelial vectorial transport of Na\(^+\) and acid/base equivalents (40, 52, 56). To date, five NHE isoforms (NHE1 to NHE5) have been cloned (3, 43, 44, 49, 50, 53). NHE1 is expressed in virtually all cell types (40), and NHE2 and NHE3 have been detected in intestine and kidney where they are targeted to the apical membrane of epithelial cells (7, 8, 9, 28, 47). NHE3 is involved in sodium absorption in the intestine, and in sodium reabsorption coupled to bicarbonate reabsorption in the kidney. NHE4 mRNA was detected in various organs including stomach, intestine, and kidney (9). In the kidney, it is located on the basolateral membrane of epithelial cells that are in contact with highly concentrated fluids. Because NHE4 is activated under hypertonic conditions, it is likely to be involved in cell volume regulation. Thus NHE1 and NHE4 are proposed to regulate cell pH and volume, respectively, whereas NHE2 and NHE3 participate in net transepithelial transport of Na\(^+\) and H\(^+\) in several absorptive epithelia.

The epithelium lining parts of the male reproductive tract is involved in active transepithelial transport of water and various solutes. Epithelial cells of the epididymis and vas deferens play a vital role in establishing the luminal environment in which spermatozoa mature and are stored (26, 33, 45). After leaving the testis, spermatozoa enter the efferent ducts, which extend from the rete testis to the epididymis. In the rat, the epididymis is composed of one convoluted tubule and is divided into several regions. The proximal region of the epididymis contains the initial segments, the intermediate zone, and the caput (or head). The corpus (or body) of the epididymis is in the middle part and connects the caput epididymis to the cauda (or tail) epididymis, located in the distal region, just before the vas deferens.

The efferent ducts are related embryologically to the kidney (27). They are remnants of the mesonephric...
kidney ducts that are closely related to renal proximal tubules. Isotonic fluid reabsorption with net sodium and chloride reabsorption occur in the efferent ducts, as in the homologous kidney proximal tubule (21, 29). During embryological development, the efferent ducts link the testis to the Wolffian duct, which differentiates to form the vas deferens. The epididymis derives from the Wolffian duct, as does the kidney collecting duct. The tubular fluid in the lumen of the epididymis undergoes considerable changes in composition as a consequence of net water, Na+, Cl− and HCO3− absorption, K+ secretion, and acidification (4, 30, 38, 51, 54). As the efferent duct fluid transits through the initial segment and intermediate zone of the epididymis, the luminal bicarbonate concentration becomes significantly lower than that of blood (38), and an acidic pH is established in all regions of the epididymis and vas deferens (18, 37, 38). These factors help to maintain spermatooza in an immotile state while they mature and are stored in the epididymis (1). Thus the development of the kidney and the excurrent duct system of the male reproductive tract are closely intertwined, and this is reflected by their similar transport properties.

Work from our laboratory has shown that a subpopulation of epithelial cells in the epididymis and vas deferens express high levels of the vacuolar type H+-ATPase on their apical membrane and subapical vesicles (11, 13, 15), and that up to 80% of net proton secretion is inhibited by bafilomycin in isolated vas deferens (11, 13). Cells that express the H+-ATPase are the narrow cells in the initial segments, and the clear cells in the caput, corpus, and cauda epididymis. These cells also contain high levels of the cytosolic carbonic anhydrase, CAII (11, 13, 22, 34), implicating the involvement of bicarbonate in the acidification process. We have recently shown the presence of the Cl/HCO3− exchanger AE2 (32) and of the electrogenic Na/HCO3− cotransporter NBC (31) on the basolateral membrane of epithelial cells lining the lumen of the epididymis and vas deferens, these proteins being expressed at higher levels in the more proximal regions (initial segments, intermediate zone, and caput epididymidis) compared with the cauda epididymidis and vas deferens. Altogether, these results indicate that bicarbonate is being reabsorbed in these tissues and that the relative contribution of various transporters depends on the epididymal regions in which acid/base transport occurs.

In the rat efferent ducts microperfused in vivo, up to 70% of net fluid reabsorption is inhibited by amiloride, indicating that Na+/H+ exchange is a major transport pathway for fluid and electrolyte reabsorption in these segments (24). In the cauda epididymidis, net sodium reabsorption is inhibited by amiloride and does not depend on luminal chloride (54). Because luminal sodium was required for the acidification process to take place in this segment (4), an apical Na/H exchanger was then proposed to be involved in this process. Altogether, these results suggest that net transepithelial transport of NaHCO3 occurs in the epididymis, as is the case for other reabsorptive epithelia, such as the kidney proximal tubule. Whereas the contribution of the apical NHE3 has been well established in the proximal tubule (7, 8, 40, 55), a recent study showed that NHE2 (−/−) mice did not have a significant renal acidification defect and that the rate of sodium-dependent proton secretion in isolated proximal tubules in vitro was comparable to control mice (20). It was then concluded that NHE2 does not mediate proximal tubule NaH antiporter activity. A recent report has shown the presence of NHE2 on the apical membrane of principal cells in some regions of the epididymis, and its absence from the initial segments (19). In the present study, we examined the potential role of NHE3 in Na-related acidification in the epididymis and vas deferens. We used an affinity-purified monoclonal antibody to localize NHE3 in the rat epididymis and vas deferens, and NHE functional activity was assessed on isolated tubules from the initial segment and cauda epididymidis perfused in vitro and loaded with the pH-sensitive dye 2′,7′-bis(carboxyethyl)-5(6′)-carboxyfluorescein (BCECF).

MATERIALS AND METHODS

Antibodies

An affinity-purified monoclonal antibody (19F5) against a fusion protein containing amino acids 702–832 of rabbit NHE3 was used. This antibody has been characterized previously (8). It is highly specific for NHE3 and does not recognize other NHE isofoms. To distinguish between principal cells and narrow and clear cells in the epididymis, an affinity-purified chicken antibody raised against the COOH-terminal 14 amino acids of the 31-kDa subunit (E subunit) of the H+-ATPase was used (14). To identify the ciliated cells in the efferent ducts, an affinity-purified rabbit antibody raised against the Cl/HCO3− AE2 exchanger (provided by Seth Alper, Beth Israel Deaconess Medical Center) was used. A goat anti-mouse IgG conjugated to FITC was used to detect NHE3, and donkey anti-chicken or goat anti-rabbit IgG conjugated to CY3 was used to detect H+-ATPase and AE2, respectively.

Immunocytochemistry

Mature male Sprague-Dawley rats were anesthetized with an intraperitoneal injection of Nembutal (0.2 ml/100 g body wt of a 50 mg/ml solution). The male reproductive organs were fixed via left ventricle perfusion with PBS (0.9% NaCl in 10 mM sodium phosphate buffer, pH 7.4) followed by 150 ml of fixative solution containing 4% paraformaldehyde, 10 mM sodium periodate, 75 mM lysine, and 5% sucrose in 0.1 M sodium phosphate buffer, pH 7.4, and stored at 4°C in PBS containing 0.02% sodium azide. For cryostat sectioning, the epididymis was separated into two parts, one including the initial segments, the intermediate zone, the caput epididymidis, and the other including the distal corpus, the cauda epididymidis, and the proximal vas deferens. Tissues were cryoprotected by immersion in 30% sucrose/PBS for at least 4 h, mounted in Tissue-Tek.
Mature Sprague-Dawley rats were anesthetized with Nembutal as described above. Epididymis was harvested and kept either in a cold preservation fluid containing (in mM) 56 Na$_2$HPO$_4$, 13 NaH$_2$PO$_4$, and 140 sucrose, pH 7.4, or they were kept at 37°C in a physiological solution (control solution in Table 1) bubbled with O$_2$. Tubules were dissected from the distal initial segments or the proximal cauda epididymidis by using fine forceps. They were then transferred into the perfusion chamber on the stage of a Zeiss inverted microscope, and peritubular and luminal perfusion were performed, as described previously for kidney proximal tubules (10, 35).

**Table 1. Solution constituents**

<table>
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<tr>
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<th>Ctl</th>
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<tbody>
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<td>K citrate</td>
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</tr>
<tr>
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<td></td>
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<td>Na cyclamate</td>
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All constituents are in mM. Solutions were bubbled with 100% O$_2$. Ctl, control; NMDG-Cl, N-methyl-D-glucamine chloride. pH was adjusted to 7.4 with TRIS/HEPES buffer.

**pH$_i$ Measurements**

pH$_i$ was measured by using the fluorescent dye BCECF (Molecular Probes), as described previously (5, 10). The ace- toxymethyl ester form of the probe was added to the luminal perfusate at a final concentration of 5 μM, and loading was allowed for 5–10 min at 30°C. Dual excitation at 450 and 500 nm was performed by using a PTI spectrofluorometer. Fluorescent light emitted at 530 nm and corrected for background was detected from the epithelial cells lining the tubule lumen. For each experiment, calibration of the dye was performed after equilibration of the tubule in solutions containing 120 mM potassium and 10 μM nigericin at pH 6.75, 7.0, and 7.25. pH$_i$ was estimated from the ratio of the emitted light at the two excitation wavelengths.

After the steady-state pH$_i$ was monitored under control conditions, an acute acid load was induced by a pulse of 20 mM NH$_3$/NH$_4$ in the luminal solution (replacing N-methyl-D-glucamine), as described previously (39). NHE function was assayed as the ethylisopropyl amiloride (EIPA)-dependent inhibition of the initial rate of pH$_i$ recovery (dpH$_i$/dt). dpH$_i$/dt was estimated from the linear portion of the pH$_i$ recovery by using the linear regression function in Excel 5.0. These experiments were performed in the absence of nical carbonate in the peritubular and luminal perfusates to minimize the contribution of HCO$_3^-$-transporting mechanisms in pH$_i$ recovery.

**Statistics**

Data are expressed as means ± SE, where n refers to the number of tubules analyzed. Two tailed paired t-tests were performed by using Excel 5.0.

**RESULTS**

**Immunolocalization of NHE3**

Immunocytochemistry on cryostat epididymis sections using horseradish peroxidase revealed that NHE3 is located in the apical membrane of epithelial cells and that its level of expression varies with the epididymal region examined (Fig. 1, A and B). NHE3 is also expressed by connective tissue. As shown in Fig. 1A, proximal and middle initial segments are negative for NHE3 (see Refs. 2 and 25 for nomenclature). NHE3
staining starts to appear in the distal initial segment and intermediate zone, and a strong staining is observed in the proximal caput epididymidis, whereas the distal caput shows a low level of NHE3 expression. The proximal corpus epididymidis also shows a weak staining for NHE3 (data not shown). Figure 1B shows the distal corpus epididymidis, the cauda epididymidis, and the proximal region of the vas deferens stained for NHE3. NHE3 expression also varies in these regions, being moderate in the distal corpus epididymidis and highest in the proximal cauda epididymidis. The distal cauda epididymidis and the vas deferens show no detectable levels of NHE3. Spermatozoa in all regions of the epididymis, also, do not show expression of NHE3.

Higher magnification immunofluorescence microscopy revealed the same pattern of NHE3 expression in various regions of the epididymis and vas deferens. The efferent ducts, which connect the testis to the epididymis, express high levels of NHE3 on their apical membrane (Fig. 2). These segments contain two cell types, the ciliated cells, which express high levels of the basolateral $\text{Cl}^-/\text{HCO}_3^-$ exchanger AE2 (32), and the nonciliated cells. As shown in Fig. 2, the nonciliated cells show a bright apical staining for NHE3, and cells that are positive for AE2 do not express NHE3. In the epididymis, the proximal and middle initial segments were negative (not shown), but the distal initial segment (Fig. 3A) and the intermediate zone (Fig. 3C) showed a strong staining of the apical membrane of most epithelial cells. Double labeling for $\text{H}^+\text{-ATPase}$ (Fig. 3, B and D) revealed the presence of a few narrow cells (positive for $\text{H}^+\text{-ATPase}$) in these segments. Due to the presence of long principal cell apical microvilli (or stereocilia), which extend extensively into the tu-

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**Fig. 1.** Immunocytochemical localization of Na$^+$/H$^+$ exchanger (NHE3) on cryostat sections of rat epididymis using horseradish peroxidase. Each figure is a montage of 20 individual images taken with a $\times4$ objective on a Nikon E800 microscope. Images were assembled by using IPLab Spectrum software. A: proximal portions of the epididymis including the initial segments, intermediate zone, and caput epididymidis. Proximal (PIS) and middle (MIS) initial segments are negative for NHE3, the distal initial segment (DIS) shows a moderate apical staining, the intermediate zone (IZ) and proximal caput epididymidis (PCap) are strongly stained, and the distal caput epididymidis (DCap) shows a very weak apical staining. B: distal portions of the epididymis including the distal corpus (DCor) and the cauda epididymidis. A moderate staining is seen in the DCor, and no staining is seen in the distal cauda epididymidis (DCau). The highest staining is observed in the proximal cauda epididymidis (PCau). Connective tissue is stained for NHE3, and spermatozoa are negative. Bar = 1 mm.
bule lumen, it was difficult to determine whether narrow cells also expressed NHE3 on their apical membrane. However, careful examination of sections at higher magnification by confocal microscopy clearly showed that H^+-ATPase-rich cells do not express NHE3 (Fig. 4).

A strong apical staining was detected in principal cells of the proximal caput epididymidis (Fig. 5A), and a weaker NHE3 staining was observed in the distal caput epididymidis (Fig. 5C). In these regions, H^+-ATPase-rich cells are wider and principal cell microvilli are shorter than in the more proximal regions.
Double labeling for the H⁺-ATPase revealed that NHE3 is restricted to principal cells (Fig. 5, A–D). Confocal microscopy confirmed that NHE3 staining was absent from H⁺-ATPase-rich cells in these regions (not shown). NHE3 labeling became progressively weaker in the corpus epididymidis (data not shown) and reintensified in the proximal cauda epididymidis, where principal cells showed a strong apical staining (Fig. 6 A–C). In the transition region between the proximal and distal cauda epididymidis, some principal cells became negative for NHE3 (Fig. 6A, arrows), and no staining was detected in the principal cells of the distal cauda epididymidis and the vas deferens (Fig. 6B). Figure 6C shows that H⁺-ATPase-rich cells do not express NHE3 in the proximal cauda epididymidis.

**Na⁺/H⁺ Exchange Functional Activity in Isolated Tubules**

**Distal initial segment and intermediate zone.** In a first series of experiments, the steady-state pHᵢ measured in 10 tubules isolated from the distal initial segment or the intermediate zone was 7.06 ± 0.04 under control conditions. As shown in Fig. 7A, addition of NH₄⁺/NH₃⁺ in the lumen produced a rapid intracellular alkalization due to the influx of NH₃ into the cells. On washout of NH₄⁺/NH₃⁺, a marked acidification was induced as intracellular NH₃⁺ dissociated into NH₃ and H⁺, followed by a spontaneous pHᵢ recovery. After pHᵢ has returned to its control value, the Na⁺/H⁺ exchanger inhibitor, EIPA, was added to the luminal perfusate at a final concentration of 50 μM for 5 min. A second pulse of NH₄⁺/NH₃⁺ was then applied in the continuous presence of EIPA. On average, dpHᵢ/dt was 0.14 ± 0.01 under control conditions and was reduced to 0.07 ± 0.01 in the presence of EIPA (51% inhibition; n = 10; P < 0.005; Fig. 7B). Control experiments were also performed in which two consecutive pulses of NH₄⁺/NH₃⁺ were induced without addition of EIPA (not shown). In this series of five tubules, dpHᵢ/dt was 0.12 ± 0.02 and 0.15 ± 0.02 pH unit/min in response to the first and second pulse of NH₄⁺/NH₃⁺, respectively (P = not significant [NS]).

To determine whether the Na⁺/H⁺ exchange activity measured in isolated tubules from the distal initial segments was generated by an apical or basolateral Na⁺/H⁺ exchanger, the luminal sodium was removed and pHᵢ and dpHᵢ/dt were measured. As shown in Fig. 8, removal of sodium (replaced by N-methyl-D-glucamine; Na = 0 in Table 1) in the luminal perfusate induced a progressive intracellular acidification from an initial value of 7.27 ± 0.04 to reach 6.97 ± 0.07 after 5 min (n = 5, P < 0.005). In this series of experiments, three pulses of NH₄⁺/NH₃⁺ were performed: the first and third were applied before and after the sodium removal period (controls), and the second was applied in the absence of sodium. The dpHᵢ/dt values from the two control pulses were averaged and compared with the value measured under sodium-free conditions. dpHᵢ/dt was 0.14 ± 0.02 pH unit/min under control conditions, and was reduced to 0.06 ± 0.01 pH unit/min in the absence of luminal sodium (52% inhibition; n = 5; P < 0.0005). Therefore, both EIPA and luminal sodium removal induced an inhibition of dpHᵢ/dt of the same magnitude, indicating that an apical Na⁺/H⁺ exchange activity was being detected.

Our results showing strong apical staining for NHE3, together with the previously reported absence of NHE2 in the initial segments (19), pointed toward a role for NHE3, and not NHE2, in isolated tubules from the distal initial segment. In an attempt to identify the NHE isoform responsible for the apical Na/H exchange
activity, we used the inhibitor HOE694 (Hoechst, Frankfurt, Germany). Previous reports have shown that, at a concentration of 50 μM, HOE694 should reduce most of the NHE2 activity and should not affect NHE3 (23). To our surprise, HOE694 inhibited $dpH_i/dt$ by 51 ± 12% (n = 4; $P < 0.005$; Fig. 9), and EIPA, when added after HOE694, did not induce any additional reduction of $dpH_i/dt$ (48 ± 2.4% inhibition compared with control value: $n = 4$; $P < 0.005$ vs. control; $P = \text{ns}$ vs. HOE694).

**Proximal cauda epididymidis.** Na$^+$/H$^+$ exchange activity was also measured on tubules isolated from the proximal cauda epididymidis. In this series of 11 experiments, the control steady-state pH$_i$ was 7.19 ± 0.05. $dpH_i/dt$ in response to an NH$_3$/NH$_4^+$-induced acid load was 0.23 ± 0.02 pH unit/min under control conditions and was reduced to 0.12 ± 0.01 pH unit/min (45% inhibition; $P < 0.005$; Fig. 10) by 50 μM EIPA. Control experiments including two consecutive NH$_3$/NH$_4^+$ pulses without EIPA were also performed. $dpH_i/dt$ were 0.25 ± 0.05 and 0.20 ± 0.03 pH unit/min in response to the first and second pulse of NH$_3$/NH$_4^+$, respectively ($n = 5$; $P = \text{NS}$).

**DISCUSSION**

Both pH and bicarbonate regulate the motility of spermatozoa (1, 41, 42, 48). The establishment of an
acidic pH and of a low HCO$_3^-$ concentration in the luminal fluid of the epididymis and vas deferens (18, 37, 38) contribute to the maintenance of sperm in a quiescent state while they mature and are stored in these organs (26, 33, 45). Our laboratory has shown that narrow and clear cells express high levels of the vacuolar H$^+$-ATPase in their apical pole and are responsible for the majority of proton secretion in isolated vas deferens (11, 13, 15). In addition, an earlier study has shown that acidification was dependent on...
the presence of sodium in the lumen of cauda epididymis perfused in vivo (4), and an Na+/H+ exchanger was proposed to participate in this process. The purpose of the present study was to determine whether the Na+/H+ exchanger NHE3 was involved, in addition to the H+ -ATPase, in the acidification of the epididymal lumen.

NHE3 plays a major role in acid-base balance and Na-fluid homeostasis (46). It is highly expressed on the apical membrane of kidney proximal tubules and intestinal epithelial cells, where it is involved in NaCl and HCO$_3$$_3$ absorption (7, 8, 28, 40, 52, 56). In view of the transepithelial Na$^+$ and HCO$_3$$_3$ reabsorption and H$^+$ secretion mechanisms that take place in the epididymis and vas deferens, we investigated whether this isoform is present in these tissues. By using a highly purified monoclonal antibody, we found that NHE3 is expressed in nonciliated cells of the efferent ducts and in principal cells of the epididymis, where it is located on the apical membrane. Narrow and clear cells, which were identified by their high expression of the vacuolar H$^+$-ATPase, do not contain NHE3. Interestingly, the level of expression of NHE3 varies in different regions of the epididymis. In the proximal parts of the epididymis, NHE3 is most abundant in the distal initial segment, the intermediate zone, and the proximal caput epididymidis. In the distal caput and the corpus epididymidis, NHE3 is barely detectable. In the distal regions of the epididymis, NHE3 is expressed in the proximal cauda epididymidis only, being absent from the distal cauda epididymidis and vas deferens.

Functional assays using BCECF on isolated tubules from the initial segment/intermediate zone region, and from the cauda epididymidis showed that Na$^+/H^+$ exchange accounts for $\sim$50% of acid extrusion, in the nominal absence of extracellular HCO$_3$$_3$. In the initial segments, pH$_i$ recovery in response to an acid load was greatly reduced in the absence of luminal sodium. The inhibition induced by the removal of Na$^+$ was similar to that induced by EIPA (in the presence of Na$^+$) indicating that the EIPA-sensitive pH$_i$ recovery was due to the activity of an apical Na$^+/H^+$ exchanger. We then tried to identify pharmacologically the NHE isoform responsible for this activity. Because the initial segments were reported to be negative for NHE2 by immunocytochemistry (19), NHE3 was the most likely isoform to be involved in this region. However, HOE694, used at a concentration that was expected to inhibit most NHE2 activity but not NHE3 (23), inhibited all the EIPA-sensitive pH$_i$ recovery. This result can be interpreted in several ways: 1) NHE2 is present in the initial segments, but has so far remained undetected; 2) an NHE3 splice variant, sensitive to HOE694, is expressed in the epididymis; and 3) epididymal posttranslational modification confers to NHE3 sensitivity to HOE694. NHE2 might have remained undetected by immunocytochemistry in the initial segments for different reasons, including reduced immunoreactivity by masking of the antigenic site, due to protein-protein interaction. This has been shown for some membrane proteins, such as NHE3, the detection of which is reduced by its interaction with megalin (6). It is, therefore, possible that a lower NHE2 immunoreactivity might have precluded its detection in the initial segments compared with the rest of the epididymis. In that case, the HOE694 effect observed in the present study would be due to inhibition of NHE2. Alternatively, sensitivity of NHE3 to HOE694 has been recently shown in the main pancreatic duct (36). In this study, most of the NHE3 activity was inhibited by 50 $\mu$M HOE694 in pancreatic ducts isolated from normal and NHE2 (-/-) mice, whereas no inhibition of the kidney proximal tubule NHE3 was observed. The authors concluded that sensitivity to HOE694 (and possibly other amiloride analogs) cannot be used in all cases to discern the NHE isoform expressed in a given location.

![Fig. 9](http://ajprenal.physiology.org/)

Fig. 9. Histogram showing the mean effect of 50 $\mu$M HOE694 and 50 $\mu$M EIPA on pH$_i$ recovery in response to an acute acid load in 4 tubules isolated from the DIS. HOE694 significantly inhibited pH$_i$ recovery, and EIPA, when added after HOE694, did not induce any additional reduction. ($^*$ P < 0.005 vs. control; $P = NS$ vs. HOE694).

![Fig. 10](http://ajprenal.physiology.org/)

Fig. 10. Histogram showing the mean effect of 50 $\mu$M EIPA on pH$_i$ recovery in response to an acute acid load in 7 tubules isolated from the cauda epididymidis. EIPA significantly reduced the rate of pH$_i$ recovery ($^*$ P < 0.005).
tissue or cell type.” It is, therefore, conceivable that, similar to the pancreatic duct, an HOE694-sensitive NHE3 is expressed in the initial segments of the epididymis. Additional experiments using NHE2 (−/−), NHE3 (−/−), and double knockout mice will be required to answer these questions.

The high levels of NHE3 in the proximal regions of the efferent duct (efferent ducts, initial segment, intermediate zone, and proximal caput epididymidis) correlate with the very low concentration of HCO₃⁻ that is reached in the caput epididymidis (38). In the epididymis, NHE3 expression also correlates with high levels of the electrogenic Na-HCO₃ cotransporter NBC and of the Cl/HCO₃⁻ exchanger AE2 in the basolateral membrane of epithelial cells in these regions (31, 32). Although no direct evaluation of the role of NHE3 in transepithelial transport was performed in the present study, the polarized expression of this proton-extruding protein in the apical membrane and of various HCO₃⁻ transporters in the basolateral membrane indicates that the initial segments, intermediate zone, and proximal caput epididymidis are highly specialized for net HCO₃⁻ reabsorption. Effenter ducts reabsorb the majority of the fluid that originates from the testis, and a previous study has shown that amiloride inhibits up to 70% of fluid reabsorption in these segments (24). These results, together with our data, suggest that NHE3 might participate in fluid and electrolyte reabsorption in the efferent duct.

NHE3 can also participate in net sodium reabsorption in various regions of the epididymis. NHE3 is expressed in the proximal cauda epididymidis (present study) and Na⁺ reabsorption is inhibited by amiloride in the perfused cauda epididymidis (54). However, NHE2 has also been localized on the apical membrane of epithelial cells of the cauda epididymidis (19), and the Na⁺/H⁺ exchange activity that we measured in this segment might reflect NHE2 activity, in addition to NHE3. The apparent sensitivity of the epidymial NHE3 to HOE694 precludes the use of this inhibitor (or possibly other amiloride analogs) to determine the relative contribution of these two isoforms in this segment. Both NHE2 and NHE3 might, therefore, contribute to net HCO₃⁻, Na⁺, and water absorption in this region of the epididymis. Na⁺-dependent proton secretion is not altered in kidney proximal tubules (20) and main pancreatic ducts (36) from NHE2 (−/−) mice compared with normal animals, and the role of NHE2 in these organs remains to be elucidated. Further experiments using NHE2 (−/−) and NHE3 (−/−) mice will be required to determine the relative contribution of these transporters in the epididymis.

Overall, with the exception of the corpus epididymidis, NHE3 is abundant in segments of the epididymis that contain fewer H⁻-ATPase-rich cells, and is not detectable in the distal cauda epididymidis, where H⁻-ATPase-rich cells are numerous. In the distal cauda epididymidis, H⁻-ATPase-rich cells are poised to play a central role in the final acidification mechanisms in the regions where spermatozoa are stored. Our laboratory has shown that in the vas deferens, H⁺-ATPase-rich cells are responsible for up to 80% of proton secretion (13). In addition, the absence of NHE3 in the vas deferens correlates with our previous results showing no effect of amiloride on net proton secretion in this segment (17).

In summary, our study shows a high expression of the Na⁺/H⁺ exchanger, NHE3, in efferent ducts, initial segments, intermediate zone, proximal caput, and proximal cauda epididymidis, whereas NHE3 was not detectable in the distal cauda epididymidis and vas deferens. These results suggest that transepithelial acid/base transport involves different sets of proteins in various regions of the efferent duct system, NHE3 being involved in HCO₃⁻ reabsorption in the proximal regions of the epididymis, and the vacular H⁺-ATPase participating in net acid secretion in the distal portions of the epididymis.

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