Cloning and Characterization of a Na\(^+\)-driven Anion Exchanger (NDAE1)

A NEW BICARBONATE TRANSPORTER*

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Regulation of intra- and extracellular ion activities (e.g. H\(^+\), Cl\(^-\), Na\(^+\)) is key to normal function of the central nervous system, digestive tract, respiratory tract, and urinary system. With our cloning of an electrogenic Na\(^+\)/HCO\(_3\) cotransporter (NDC), we found that NBC and the anion exchangers form a bicarbonate transporter superfamily. Functionally three other HCO\(_3\) transporters are known: a neutral Na\(^+\)/HCO\(_3\) cotransporter, a K\(^+\)/HCO\(_3\) cotransporter, and a Na\(^+\)-dependent Cl\(^-\)/HCO\(_3\) exchanger. We report the cloning and characterization of a Na\(^+\)-coupled Cl\(^-\)/HCO\(_3\) exchanger and a physiologically unique bicarbonate transporter superfamily member. This Drosophila cDNA encodes a 1050-amino acid membrane protein with both sequence homology and predicted topology similar to the anion exchangers and NBCs. The mRNA is expressed throughout Drosophila development and is prominent in the central nervous system. When expressed in Xenopus oocytes, this membrane protein mediates the transport of Cl\(^-\), Na\(^+\), H\(^+\), and HCO\(_3\) but does not require HCO\(_3\). Transport is blocked by the stilbene 4,4\(^{\prime}\)-disothiocyanodihydrostilbene-2,2\(^{\prime}\)-disulfonates and may not be strictly electroneutral. Our functional data suggest this Na\(^+\)- driven anion exchanger (NDAE1) is responsible for the Na\(^+\)-dependent Cl\(^-\)/HCO\(_3\) exchange activity characterized in neurons, kidney, and fibroblasts. NDAE1 may be generally important for fly development, because disruption of this gene is apparently lethal to the Drosophila larva.

Ionic homeostasis is the key to normal function of most biological systems. This regulation is especially important for tissues with highly specialized functions, such as the central nervous system (CNS), digestive tract, respiratory tract, and urinary system. Active transport of ions by ATPases (pumps) maintains ionic gradients and aid ion channels in “setting” the membrane potential. Secondary active transporters make use of one or more aspects of the membrane electrochemical gradient to specifically move ions and nutrients into and out of cellular compartments.

With our cloning of an electrogenic Na\(^+\)/HCO\(_3\) cotransporter (NBC; i.e. SLC4A42), we found that NBC and the anion exchangers (AEs; i.e. SLC4A1-SLC4A3) form a bicarbonate transporter superfamily (BTS) (1–3). More recently three groups have reported unique full-length cDNAs, which are additions to the BTS: NBC-2 from retina (13), an electroneutral NBC (NBCn1) (14), and NBC-3 (SLC4A7) from muscle (15). Functional data for NBC-2 have not been reported. NBCn1 is an electroneutral Na\(^+\)/HCO\(_3\) cotransporter that is partially blocked by DIDS (14). NBC-3 is currently characterized as a DIDS-insensitive, 5-(N-ethyl-N-isopropyl) amidol-sorcinol-sensitive, Na\(^+\)/HCO\(_3\) cotransporter (15) whose electrical nature is not yet known. It is presently unclear whether these clones arise from separate genes or are splicing isoforms. Of the NBC clones reported, none are Cl\(^-\)-dependent or transport Cl\(^-\).

Physiologically two other HCO\(_3\) transporters are known, a K\(^+\)/HCO\(_3\) cotransporter (5) and a Na\(^+\)-dependent Cl\(^-\)/HCO\(_3\) exchanger (6, 7). Here we report the cloning and characterization of a cation-coupled Cl\(^-\)/HCO\(_3\) exchanger and a physiologically unique BTS member from Drosophila. When expressed in Xenopus oocytes, this membrane protein mediates the transport of Cl\(^-\), Na\(^+\), H\(^+\), and HCO\(_3\) but does not require HCO\(_3\). Transport is blocked by the stilbene DIDS and may not be strictly electroneutral. Our expression data suggest this Na\(^+\)- driven anion exchanger (NDAE1) (GenBank\textsuperscript{TM} accession number AF047468) is responsible for the Na\(^+\)-dependent Cl\(^-\)/HCO\(_3\) exchange activity characterized in neurons (6–8), kidney (9, 10), and fibroblasts (11).

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| 2,2\(^{\prime}\)-disulfonate; NDAE1, Na\(^+\)- driven anion exchanger; AE, anion exchanger; bp, base pair(s); RT, reverse transcriptase; PCR, polymerase chain reaction; TM, transmembrane span; pH\(_i\), intracellular pH; eCl\(_i\), intracellular Cl\(^-\) activity; αNa\(_i\), intracellular Na\(^+\) activity; UTR, untranslated region. |
| The abbreviations used are: CNS, central nervous system; NBC, electrogenic Na\(^+\)/HCO\(_3\) cotransporter (i.e. SLC4A4); BTS, bicarbonate transporter superfamily; DIDS, 4,4\(^{\prime}\)-disothiocyanodihydrostilbene-2,2\(^{\prime}\)-disulfonate; NDAE1, Na\(^+\)- driven anion exchanger; AE, anion exchanger; bp, base pair(s); RT, reverse transcriptase; PCR, polymerase chain reaction; TM, transmembrane span; pH\(_i\), intracellular pH; eCl\(_i\), intracellular Cl\(^-\) activity; αNa\(_i\), intracellular Na\(^+\) activity; UTR, untranslated region. |
| The electrogenic NBC is currently designated by several nomenclatures in the literature: NBC1, kNBC, pNBC, hhNBC, and SLC4A4 (see Ref. 3 for a detailed explanation). SLC4A4 is the human gene designation indicating “solute carrier family 4A, member 4” by the human genome nomenclature. The clones that are currently referred to as NBC2, mNBC3, and NBC-1 are likely splice variants of the same gene; however, this has not been explicitly demonstrated. Currently NBC2 is given a designation of SLC4A6 and mNBC3 as SLC4A7. Another apparently distinct human cDNA, SLC4A8, was deposited in GenBank\textsuperscript{TM} (AF069512) but has not yet been functionally characterized. |

24552 This paper is available on line at http://www.jbc.org
Cloning—We identified a *Drosophila* expressed sequence tag (AA567741, deposited by the Berkeley *Drosophila* Genome Project) with similarity to both the AE s and NBCs. We obtained this *Drosophila* clone (Research Genetics, St. Louis, MO) and sequenced it (W. M. Keck Biotechnology Resource Laboratory, New Haven, CT). This 3225-base pair clone has an initial Met followed by a 3090-base pair reading frame flanked by 104 bp and 9 bp, respectively. This *Drosophila* cDNA encodes for the assembled genomic contig (encoding the EcoRI/HindIII fragment of the *pSport 2* construct), according to the manufacturer's instructions (Life Technologies, Inc.) with *Drosophila* poly(A)^+^ RNA. Using *Drosophila* NDAE1-specific primers, ExTaq polymerase (Panvera, Madison, WI), and dNTPs, we performed PCR with 30 cycles of 94 °C (30 s), 55 °C (45 s), and 72 °C (45 s). Products were verified with 0.65% agarose/Tris borate EDTA gel. The gel was Southern blotted onto Zeta-probe (Bio-Rad) and detected using random hexamer-priming with Biotin-HighPrime® (Roche Molecular Biochemicals), recorded on x-ray film, and verified with a 0.65% agarose/Tris borate EDTA gel. The gel was Southwestern blot analysis of poly(A)^+^ RNA from *Drosophila* developmental stages and body segments as previously reported (1) and the alignment using the Clustal method and the PAM250 residue weight table (DNA digitized using Adobe PhotoShop (Fig. 2).)

**Statistical Analysis**—Values quantitated are indicated as the mean ± S.E. Ion activities between control and NDAE1 oocytes were shown by a two-tailed t test to have a significance of p < 0.016 or less.

**RESULTS**

**Characterization of the NDAE1 cDNA and Predicted Protein**—cDNA sequencing of our clone revealed a single, long open reading frame flanked by 5'- and 3'-UTRs (UTRs, 426 and 104 bp, respectively). This *Drosophila* cDNA encodes a 1030-amino acid membrane protein with both sequence homology and predicted topology similar to both the AE s and NBCs. The predicted protein is 43% similar to the cloned NBCs and 32% similar to the AE s (Fig. 1, a and b). Although the NDAE1 hydropathy plot (Fig. 1b) is similar to those of the B Ts members, it is most similar to the AE s. A dendrogram of the published B Ts sequences (Fig. 1c) implies that NDAE1 forms a new branch of this superfamily. Our *Drosophila* topology model (Fig. 1d) predicts (i) intracellular NH2 and COOH termini, (ii) 12 transmembrane spans (TMs), (iii) a central exofacial loop with putative N-glycosylation sites, and (iv) multiple putative phosphorylation sites.

Recently the complete sequence of the *Drosophila* genome was reported (20). Although a predicted gene product "CG4675" in two forms (AAF52496, alt 1 and 2 for proteins and AE003616 for the assembled genomic contig) encoding the *nda1* gene was identified, the sequence analysis is not completely accurate. The predicted protein sequences are missing thirteen NH2-terminal amino acids (MAEKKEYIELPW) partly encoded from an additional 5'-intron. CG4675-alt 2 contains a 69-amino acid insertion (amino acids 32–100 of the NH2-truncated protein), which we have not found present in *Drosophila* mRNA. A second *Drosophila* gene and protein have homology to the B Ts family (CG8177). Using a pileup analysis, gene product CG8177 (GenBank™ accession number AAQF0207) is about 32% identical to NDAE1 and the NBCs, but about 34–40% identical to the AE s. Though CG8177 apparently encodes a HCO3− transporter protein, future transport experiments will be needed to determine the actual function.

**Expression Profile of NDAE1 mRNA**—Next, we determined the location of NDAE1 mRNA in *Drosophila*. Using Northern blot analysis of poly(A)^+^ RNA, we were unable to detect NDAE1 mRNA in embryos, isolated adult heads, or body parts. However, by RT-PCR we could detect NDAE1 mRNA in heads as well as several embryonic stages (Fig. 2a). A Southwestern hybridization to NDAE1 mRNA in whole mount *Drosophila* embryos (Fig. 2, b and c) illustrates that NDAE1 is present during embryogenesis. CNS staining is apparent throughout embryogenesis (Fig. 2, b and c). Staining of the gut primordium and mesoderm is evident in stage 6/7 (Fig. 2b). Staining of a specific subset of cells in the CNS is detectable by late embryogenesis (Fig. 2c) as is staining of the anal plate (not shown), i.e. the ionophore 1 mixture B (Fluka) and backfilled with 0.15 M NaCl. Electrodes were connected to a high impedance electrometer (WPI-FD223 for intracellular pH (pH), intracellular Cl− activity (aCl), or intracellular Na+ activity (aNa), and Vm (experiments) , and digitized output data were acquired by computer. All ion-selective microelectrodes had resistance of 54 to −57 mV/decade ion concentration (or activity). pH electrodes were calibrated at pH 6.0 and 8.0; Cl− and Na+ electrodes were calibrated with unbuffered 10 and 100 mM NaCl (ionic strength was not identical). Selectivity of Cl− was checked using unbuffered 100 mM NaHCO3 and for Na+ using 100 mM KCl. Na+ electrodes were greater than 50-fold selective for Na+ (19) and Cl− electrodes were at least 5-fold selective versus HCO3−. For voltage-clamp experiments (Warner Inst. Co., Oocyte Clamp), electrodes were filled with 3 M KCl/ligar and 3 M KCl and had resistances of 0.2–0.5 MΩ. Oocytes were clamped to −60 mV and stepped from −160 to +60 mV in 20 mV steps; the resulting data were filtered at 5 kHz (8 pole Bessel filter, Frequency Devices) and sampled at 1 kHz as previously reported (19). Data were acquired and analyzed using Pulse and PulseFit (HEKA Instruments, Germany).

**EXPERIMENTAL PROCEDURES**

**Northern Analysis and RT-PCR Protocol**—We isolated poly(A)^+^ RNA from *Drosophila* developmental stages and body segments as described previously (1). We used 2 μg of poly(A)^+^ RNA from these stages for denaturing electrophoresis and electroblotting. The NDAE1-cDNA was random primed and (α-^32^P)-labeled. Hybridization overnight at 60 °C in ExpressHyb (CLONTECH) followed by low stringency washing (42 °C with 2× SSC) did not result in discrete hybridization. Reverse transcription was performed using SuperScript RT kit according to the manufacturer’s directions (Life Technologies, Inc.) with PCR of our clone revealed a single, long open reading frame flanked by 5'- and 3'-UTRs (UTRs, 426 and 104 bp, respectively). This *Drosophila* cDNA encodes a 1030-amino acid membrane protein with both sequence homology and predicted topology similar to both the AE s and NBCs. The predicted protein is 43% similar to the cloned NBCs and 32% similar to the AE s (Fig. 1, a and b). Although the NDAE1 hydropathy plot (Fig. 1b) is similar to those of the B Ts members, it is most similar to the AE s. A dendrogram of the published B Ts sequences (Fig. 1c) implies that NDAE1 forms a new branch of this superfamily. Our *Drosophila* topology model (Fig. 1d) predicts (i) intracellular NH2 and COOH termini, (ii) 12 transmembrane spans (TMs), (iii) a central exofacial loop with putative N-glycosylation sites, and (iv) multiple putative phosphorylation sites.

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**Fig. 1. NDAE1 sequence and model.**

*Panel a,* multiple sequence alignment of NDAE1 (AF047468), human muscle NBC3 (AF047033), human retinal NBC2 (AB012130), human pancreas NBC (AF011390), and rat cardiac AE3 (A42497). NBCs are as follows: *Ambystoma* kidney NBC (aNBC), AF001958; rat kidney NBC (rkNBC), AF004017; human heart NBC (hhNBC), AF069510; and human kidney NBC (hkNBC), AF007216. In the multiple sequence alignment, identical amino acids across all five sequences are highlighted in black and in reverse type. Similar functional groups across all five sequences are in gray highlight and black type. Similar amino acids are defined by six groups: DN, EQ, ST, KR, FYW, and LIVM.

*NDAE1 predicted TMs,* i.e., 12 hydrophobic regions in *b* are indicated by brackets and a numbered line over the sequence. *b,* hydropathy plot of NDAE1. Predicted TMs are numbered 1–12. The bar between TM 5 and 6 indicates the location of the predicted extracellular loop with...
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FIG. 2. NDAE1 expression in Drosophila. We designate the Drosophila gene ndae1 and the protein NDAE1. By in situ hybridization we localized ndae1 to region 28A on Drosophila polytene chromosome 2L (not shown). a, Southern blot illustrating RT-PCR of Drosophila tissues and rNDAE1. NDAE1 gene-specific primers were used to amplify ~750-bp fragments from RT reactions of Drosophila stage and tissue poly(A)+ RNA, with NDAE1 and rNDAE included as positive and negative PCR controls, respectively. The male and female lanes are thorax without heads. The “control” lane is unrelated DNA and the “water” lane contained no template. Products are obvious in Drosophila embryos and tissues. Southern blotting showed that all of the ethidium bromide-stained NDAE1 bands hybridized authentic, biotin-labeled NDAE1 probes. b–e, in situ hybridization of NDAE1 RNA in Drosophila embryos. Drosophila embryos were fixed and probed for NDAE1 mRNA using a single-stranded, digoxigenin-labeled antisense NDAE1 RNA probe. In stage 6 embryos (b), staining was detectable in the cephalic furrow, gut primordium (arrow), all mesoderm, and some ectoderm. c, stage 16–17 embryo. There was strong staining in a subset of CNS cells and possibly some peripheral nervous system. d, e, in situ hybridization using sense RNA probes at equivalent Drosophila stages. d (stage 6) and e (stage 16) revealed no staining.

FIG. 3. NDAE1 transport model. Schematic illustrating ion movements through NDAE1. This model does not imply paired binding or that the exact transport pathway via NDAE1 is known. a illustrates the direction of NDAE1 transport, indicated as forward, at steady-state in the normal oocyte Ringer, ND96. Our model indicates that in comparison to controls NDAE1 oocytes at steady-state should (i) have a higher pHi, (ii) have a higher nNa, and (iii) have a lower aCl. This forward transport is observed experimentally with addition of bath HCO3− or removal of bath Cl−. b shows the direction of the transported ions for bath removal, “reverse” transport, of either Na+ or HCO3−. This reverse transport should (i) decrease pH, (ii) decrease nNa, and (iii) increase aCl. Even though “HCO3−” is shown in both models, NDAE1 does not require HCO3− to function (see Fig. 4d and legend, Fig. 5b, and “Results”).

Antisense RNA Sense RNA

Physiology of NDAE1 Expressed in Xenopus Oocytes—To evaluate the physiologic function of NDAE1, we expressed it in Xenopus oocytes. Fig. 3 is a model illustrating ion transport attributed to Na+−dependent Cl−-HCO3 exchange activity. We tested this model with oocytes expressing NDAE1. Fig. 4a shows that removal and replacement of bath Na+, Cl−, or both, with and without HCO3− does not alter pH, of a water-injected control cell. However, expression of NDAE1 elevates resting pHi by ~0.3 pH units (Fig. 4b, i.e. control = 7.27 ± 0.03 (n = 9) and NDAE1 = 7.54 ± 0.03 (n = 18). The acidification elicited by CO2/HCO3− (Fig. 4a) is markedly reduced in NDAE1 oocytes (Fig. 4b) and greatly increases intracellular [HCO3−]3 (control = 3.1 ± 0.2 mm, n = 9; NDAE1 = 7.4 ± 0.4 mm, n = 16). The higher resting pHi and elevated [HCO3−]4 are consistent with NDAE1’s role as an acid extruder, “forward” transport in Fig. 3a. Bath Na+ removal elicits a robust pHi decrease illustrating that NDAE1 is readily reversible (Fig. 3b). Subsequent removal of Cl− stops and slightly reverses the acidification, whereas readmission of Na+ in the sustained absence of Cl− triggers a rapid pHi recovery (Fig. 4b). A similar response is completely blocked by 200 μM DIDS (Fig. 4g). Our results indicate that

\[ \text{[HCO}_3^-\text{]} \text{ is calculated using the pHi obtained just before CO}_2, \text{steady-state pH in the presence of CO}_2/\text{HCO}_3^- \text{, and the Henderson-Hasselbalch equation (19, 21).}\]
Fig. 4. Physiology of NDAE1 expressed in Xenopus oocytes. Oocytes were injected with 50 nl of water or cRNA in water. a, c, and e are water-injected control oocytes. b, d, f–i are injected with 35 ng/oocyte of NDAE1 cRNA. All solutions are pH 7.5, and all HCO₃ solutions are 1.5%.
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CO$_2$/10 mM HCO$_3$–. Each panel shows the response of an oocyte to CO$_2$/HCO$_3$– addition, removal of Na$^+$, removal of Na$^+$ and Cl$^–$, and removal of Cl$^–$. a, pH, of water injected (control) oocyte. Both Na$^+$ and Cl$^–$ are removed + CO$_2$/HCO$_3$–. b, pH, of a NDAE1-injected oocyte. Similar experiment to a with a NDAE1-expressing oocyte. Starting pH$_i$ values for NDAE1-oocytes are ~0.3 pH units higher than controls as expected for a HCO$_3$– influx transporter, i.e. an acid extruder. c, aCl, of a water-injected oocyte. Note that aCl, is minimally altered by bath solution manipulations. d, aCl, of a NDAE1-injected oocyte. Non-CO$_2$/HCO$_3$– solutions are bubbled with 100% O$_2$, illustrating that NDAE1 does not require HCO$_3$– to function. Starting aCl is ~10 mlt less than control oocyte indicating basal Cl$^–$ extrusion from the NDAE1-oocytes. e, aNa, of a water-injected oocyte. The aNa, is unaltered by any of the bath solution manipulations. f, aNa of a NDAE1-injected oocyte. The steady-state aNa, is elevated in comparison to the control oocyte. g–i illustrate DIDS inhibition of ion transport via NDAE1. g, DIDS inhibition of NDAE1-mediated pH$_i$ changes. The oocyte was exposed twice to CO$_2$/HCO$_3$–, first without DIDS (not shown) and second with 200 $\mu$M DIDS. Exposure to DIDS appears to completely block NDAE1 activity, resulting in a response similar to control oocytes. h, DIDS inhibition of NDAE1-mediated aCl, changes, second pulse shown. i, using a double CO$_2$/HCO$_3$– protocol as in g, DIDS also blocks the aNa, changes. The hatched bar at the bottom right corner represents 10 min for that experiment.
function on αNa, of oocytes. Fig. 4, e and f shows representative traces from control and NDAE1 oocyte experiments, respectively, using similar solution protocols as in Fig. 4, a–d. A control oocyte (Fig. 4c) has 2.6 mV αNa, 3.1 ± 0.5 mV, n = 10), which does not change with bath ion substitutions. Fig. 4f shows that αNa, is increased to 2.5 mV in NDAE1-expressing oocytes (4.6 ± 0.3 mV, n = 10). Na⁺ is transported by NDAE1 as evidenced by (i) increased αNa, with the addition of CO₂/ HCO₃⁻, (ii) reduced αNa, with Na⁺ removal, and (iii) increased αNa, with Cl⁻ removal. Na⁺ transport via NDAE1 is blocked by 200 μM DIDS (Fig. 4i). Changes of αNa, are always in the opposite direction as αCl, changes indicating a Na⁺ for Cl⁻ exchange. As shown for both the pH, and αCl, responses, Na⁺ transport was also observed in the complete absence of HCO₃⁻ (not shown). Thus, our data indicate that this Drosophila Na⁺-dependent Cl-HCO₃ exchanger is more appropriately named a Na⁺-driven anion exchanger or NDAE1.

We noted that Cl⁻ removal or the addition of HCO₃⁻ resulted in significant depolarizations only in NDAE1 oocytes (Fig. 4b). Therefore, we voltage-clamped and used anion transport inhibitors (DIDS, diphenylamine carboxylic acid, and nifluimic acid) to evaluate the electrical nature of NDAE1 (Fig. 5). In a voltage-clamped oocyte, this depolarization is measured as an inward (negative) current. A comparison of water-injected control (Fig. 5a) and NDAE1 oocytes (Fig. 5b) illustrates that both Cl⁻ removal and HCO₃⁻ addition elicit current specific to NDAE1 expression. The reversal potential of both control (Fig. 5c) and NDAE1 oocytes (Fig. 5d) is about −20 mV. In the absence of Cl⁻, there is also a HCO₃⁻-stimulated current only in NDAE1 oocytes (Fig. 5b). This current has a linear voltage dependence (Fig. 5d). DIDS, diphenylamine carboxylic acid, and nifluimic acid block the depolarization (unclamped cell) because of Cl⁻ removal (Fig. 5e). However, the measured currents in NDAE1 oocytes are small compared with the pH, αCl, and αNa, changes. The voltage deflections and associated currents are either endogenous to the oocyte uncovered by NDAE1 activity or more likely a “leak” current through the NDAE1 transporter. Present data imply that the NDAE1 current represents a leak current rather than NDAE1 being “electrogenic”: (i) the J(ion)/J(current) ratio for Cl⁻, HCO₃⁻, and Na⁺ is > 1000; and (ii) the pH, changes are two to three times greater for NDAE1 than rkNBC, whereas the transport currents are at least 10-fold smaller (30 nA versus 300–500 nA, respectively) (19). These transporter currents (or voltage changes) would not have been detectable in snail neurons (7) or squid axons (6).

Identification of a P Element Insertion in the NDAE1 Gene—To begin investigating the role of NDAE1 in vivo, we searched the Berkeley Drosophila genome Project (BDGP) data base with the NDAE1 sequence and identified a P element insertion mutation in the vicinity of ndae1. This insertion lies 408 bases 5’ of the predicted NDAE1 initiation codon (Fig. 6a). By RT-PCR we determined that the insertion site of this P element mutation lies within the NDAE1 5’-untranslated sequence, using wild type pol(A)⁺ RNA and primers that flank the site of insertion (Fig. 6b). These data suggest that this P element disrupts ndae1. Because this P element insertion was isolated in a screen for lethal P element-induced mutations (12, 22), our data further suggest that ndae1 may be essential for viability. Detailed phenotypic and physiological analysis of this mutant will be presented elsewhere.

Expression of NDAE1 in Xenopus oocytes shows all the physiologic properties of the Na⁺-dependent Cl-HCO₃ exchanger: Cl⁻ transport, Na⁺ transport, Na⁺/HCO₃⁻ cotransport (or Na⁺-H⁺ exchange), and sensitivity to DIDS. NDAE1 does not require HCO₃⁻ and appears to be a more general anion exchanger. Our data indicate that NDAE1 exchanges Na⁺ and HCO₃⁻ (or an anion) for Cl⁻ and H⁺ (Fig. 3). Thus based on our functional studies, it is likely that NDAE1 is the Drosophila form of the Na⁺-dependent Cl⁻-HCO₃⁻ exchanger functionally identified in neurons, fibroblasts, mesangial cells, and renal tubule cells.

Physiologically, the activity of the Na⁺-dependent Cl⁻HCO₃⁻ exchanger appears to be regulated. In mesangial cells, agents such as angiotensin II, serotonin, and vasopressin, which act locally as growth factors (23), as well as epidermal growth factor and platelet-derived growth factor, stimulate ion transport activity including Na⁺-dependent Cl⁻-HCO₃⁻ exchange (10). Recently, Na⁺-dependent Cl⁻-HCO₃⁻ exchange activity was shown to increase during normal renal development (24). And, in NIH-3T3 fibroblasts, Kaplan and Boron (11) found that transformation with c-Ha-ras not only increased the activity of the Na⁺-dependent Cl⁻-HCO₃⁻ exchanger but also shifted activation to more alkaline pH values, effectively removing pH, as the transporter control mechanism. Moreover, some studies postulate that mis- or deregulation of stilbene-sensitive HCO₃⁻ transport (25) or Na⁺-H⁺ exchange (26) is involved in neoplasia. We postulate that future NDAE1 studies will elucidate the mechanisms for these regulatory observations.

As the first cloned Na⁺ and Cl⁻-coupled HCO₃⁻ transporter, NDAE1 may assist the molecular identification of still other cation- and anion-coupled HCO₃⁻ transporters. The identification of NDAE1 in Drosophila presents the opportunity to use genetic analysis and manipulation to further understand NDAE1 function and importance in vivo. Because disruption of the NDAE1 gene and loss of the protein is apparently lethal (12), NDAE1 is likely an important developmental protein. Cloning of mammalian NDAE1 homologs will provide novel insights to normal and pathologic roles they play in the CNS, circulatory system, digestive tract, respiratory tract (27), and urinary system. In the visual system, determining the localization of the NDAE1 may increase our understanding of fluid and ion transport by the ciliary body (28) and the lens (29) (e.g. glaucoma) or neuronal transmission in the retina. In the kidney, understanding NDAE1 function may lead to an understanding of how the Na⁺-driven Cl⁻-HCO₃⁻ exchange modulates neuronal activity by altering pH, αCl, and pH, (e.g. seizure disorders).

NDAE1 is also likely an important acid-base homeostatic regulator for insects, particularly for the gut. Based on our in situ hybridization, NDAE1 mRNA is present in the developing gut. The NDAE1 mRNA and the protein are likely to persist in the adult organism. The midguts of mosquito larvae (30) and lepidopteran larvae (31) are known to be extremely alkaline, i.e. pH 8–12. At least in mosquito larvae, this alkalinity is in part mediated by removal of H⁺ by a V-type ATPase (32) presumably working in concert with a yet unknown base secretory mechanism. Depending on the cellular location, an insect anion exchanger, e.g. NDAE1, could be involved in secretion of either acid or alkali, as in the α- and β-cells of the mammalian cortical collecting duct. In insect species with alkaline proximal guts, the distal gut is
responsible for returning the food stream to a neutral or acidic pH (33). Thus, it is possible that NDAE1 could have a more widespread role in insect acid-base balance. From the current data, we hypothesize that NDAE1 may aid in maintaining the high gut pH of Drosophila and potentially mosquitoes. The gut and salivary gland pH of mosquitoes, in particular, is a factor contributing to transmission of disease such as encephalitis and malaria. That is, acidic environments (pH < 7) appear to promote cellular infection (34). The high pH of the gut likely protects the vector organism. Infectivity of Plasmodium berghei, present in mice, to mosquitoes is reduced with low pH and low blood HCO₃⁻ of the mice (35).

A corollary hypothesis is that the mosquitoes’ ability to spread disease could be controlled by altering the transport activity of NDAE1. With the cloning of NDAE1 these questions may now be addressed at the genetic, molecular, and physiologic levels.

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