A new eukaryotic nutrient amino acid transporter has been cloned from an epithelium that is exposed to high voltages and alkaline pH. The full-length cDNA encoding this novel CAATCH1 (cation-anion-activated Amino acid transporter/channel) was isolated using a polymerase chain reaction-based strategy, and its expression product in Xenopus oocytes displayed a combination of several unique, unanticipated functional properties. CAATCH1 electrophysiological properties resembled those of Na\(^+\),Cl\(^-\)-coupled neurotransmitter amine transporters, although CAATCH1 was cloned from a gut absorptive epithelium rather than from an excitable tissue. Amino acids such as L-proline, L-threonine, and L-methionine elicited complex current-voltage relationships in alkaline pH-dependent CAATCH1 that were reminiscent of the behavior of the dopamine, serotonin, and norepinephrine transporters (DAT, SERT, NET) in the presence of their substrates and pharmacological inhibitors such as cocaine or antidepressants. These I-V relationships indicated a combination of substrate-associated carrier current plus an independent CAATCH1-associated leakage current that could be blocked by certain amino acids. However, unlike all structurally related proteins, CAATCH1 activity is absolutely independent of Cl\(^-\). Unlike related KAAT1, CAATCH1 possesses a methionine-inhibitable constitutive leakage current and is able to switch its narrow substrate selectivity, preferring threonine in the presence of K\(^+\) but preferring proline in the presence of Na\(^+\).

The distinction between ion-activated transporters and channels is becoming blurred. For example, the Na\(^+\) and Cl\(^-\) dependent neurotransmitter transporters, which serve substrates such as dopamine (DAT), norepinephrine (NET), serotonin (SERT), GABA (GATx), and selected amino acids (via EAATx, GLAST, GLT, GlyTx, PROT, or ASCx) act as both solute carriers and ion channels (1–6). The presence of a list of a subfamily of Na\(^+\)-Cl\(^-\)-dependent neurotransmitters and amino acids (23). The sense primer, S34 (5'-GGIAA/C/T/GTITGGA/C/G/A/G/C/T/T/C/C/3'), was based on a GNYWRF peptide motif, whereas the antisense primer, S21 (5'-IGC/A/G/T/ATIGCTCA/A/G/C/T/G/G/A/G/T/3'), was based on a YP/D/E/AIA peptide motif. Another sense primer, S32 (5'-GGIAA/C/T/GTITGGA/C/G/A/G/C/T/T/C/C/3'), a tolerated alternative to S34, was also used in conjunction with S21 antisense primer for initial screening. “Touchdown” hot start PCR conditions (22) with Taq DNA polymerase (Roche Molecular Biochemicals) and 1.5 mM MgCl\(_2\) were employed. Twenty cycles with decrementing annealing temperatures (60–46 °C, 0.7 °C steps) were followed by 30 cycles at the final annealing temperature. For each cycle, denaturation was at 92 °C for 40 s, and then a final 75 °C extension for 5 min was performed. The resulting 943-bp PCR fragment from the dsDNA library was TA-cloned into a pCR2.1 vector (Invitrogen) and used for transformation of competent Escherichia coli TOP10 cells. Twelve colonies were randomly picked, and the PCR products were excised and sequenced. Sequence analysis revealed high similarity between the cloned sequence and the one from the midgut of Manduca sexta larvae. The entire M. sexta midgut AATXR cDNA library was mass-expressed to generate plasmid dsDNA template, which was then used to generate unique target sequences that were isolated by degenerate primed PCR. An initial set of inosine-containing degenerate primers were designed to target conserved peptide motifs from invertebrate and vertebrate members of a subfamily of Na\(^+\),Cl\(^-\)-dependent transporters serving various neurotransmitters and amino acids (23). The sense primer, S34 (5'-GGIAA/C/T/GTITGGA/C/G/A/G/C/T/T/C/C/3'), was based on a GNYWRF peptide motif, whereas the antisense primer, S21 (5'-IGC/A/G/T/ATIGCTCA/A/G/C/T/G/G/A/G/T/3'), was based on a YP/D/E/AIA peptide motif. Another sense primer, S32 (5'-GGIAA/C/T/GTITGGA/C/G/A/G/C/T/T/C/C/3'), a tolerated alternative to S34, was also used in conjunction with S21 antisense primer for initial screening. “Touchdown” hot start PCR conditions (22) with Taq DNA polymerase (Roche Molecular Biochemicals) and 1.5 mM MgCl\(_2\) were employed. Twenty cycles with decrementing annealing temperatures (60–46 °C, 0.7 °C steps) were followed by 30 cycles at the final annealing temperature. For each cycle, denaturation was at 92 °C for 40 s, and then a final 75 °C extension for 5 min was performed. The resulting 943-bp PCR fragment from the dsDNA library was TA-cloned into a pCR2.1 vector (Invitrogen) and

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sequenced; the fragment was related to various transporters (23), including the KAAT1 neutral amino acid transporter (18).

Based on the unique sequence of the 943-bp fragment, a second nested PCR primer set was designed for use in subsequent screening steps. This primer set was designed specifically to exclude KAAT1, while amplifying a 328-bp segment of the MRF host cells. As the 943-bp sequence contained five potential translation sites, the T3 RNA polymerase promoter with the mMessage mMachine (Amersham Pharmacia Biotech) was used to transcribe the fragment. After transcribing the fragment, DyeDeoxy terminator and DyePrimer Cycle Sequencing protocols developed by Applied BioSystems were used to sequence the fragment. The sequencing was carried out in both directions of the plasmid using the ZAP-XR library (5 × 10^9 plaque-forming units total). After plasmid purification, SM buffer lysates were collected from the plate and screened by PCR to identify a positive 328-bp band using the S2S/S26 primers. The positive lysates were further screened by PCR using vector T3 sequencing primer with S26 antisense primer, and four clones with long 5'UTR sequences were obtained. The positive lysates were then re-screened by PCR. After three rounds of screening progressively smaller pools of clones, a positive lysate derived from plates seeded with 80 plaque-forming units was subsequently plated (~200 plaque-forming units total) on a single 10-cm dish with XLI-Blue MRF host cells, and a standard plaque lift was performed; the membrane (Hybond; Amersham Pharmacia Biotech) was probed using the 328-bp digoxigenin-labeled probe (above) hybridized under high stringency conditions (66 °C), yielding five positive plaques. Plasmid DNA from two plaques was excised in vivo and cloned. One clone containing a ~3-kilobase insert (CAATCH1) was used in all subsequent analyses including sequencing and physiological studies. The complete coding sequence of CAATCH1, as well as 5'- and 3'-UTRs, was determined by primer walking the insert between M13 sites of the phleomycin vector (Stratagene).

DNA samples were sequenced in the DNA Sequencing Core Laboratory of the Florida Interdisciplinary Center for Biotechnology Research. Sequencing was carried out in both directions of the plasmid using a DyeDeoxy terminator and DyePrimer Cycle Sequencing protocols developed by Applied BioSystems using fluorescence-labeled deoxyoligonucleotides and primers. The labeled extension products were analyzed with an Applied Biosystems model 373A DNA sequencer.

Transcription and Expression of cRNA in Oocytes—The plasmid containing full-length CAATCH1 cDNA was linearized by XhoI digestion, and capped cRNA was synthesized in vitro by using the T3 RNA polymerase promoter with the mMessage mMachine (Ambion) kit. Xenopus laevis oocytes at stage V or VI were injected with 50 nl of water or without 50 ng of cRNA and then incubated at 17 °C in Barth's saline for 3–10 days.

Electrophysiology—Injected oocytes were superfused (22 °C) with modified ND96 medium (98 mM NaCl, 2 mM KCl, 1 mM MgCl2,1 mM CaCl2, 10 mM TAPS plus N-methyl-d-glucamine (NMG), pH 8.0) at a constant rate (~3 ml/min unless otherwise noted) using a peristaltic pump. The pH of the medium was 8.0 unless otherwise noted. To determine the cationic specificity of CAATCH1, Na+ replaced by K+, N-methyl-d-glucamine−, choline+, Rb+, or Li+ in all buffer salts. To determine its anionic specificity, chloride− was completely replaced by Cl− in all buffer salts. Transmembrane currents were measured in intact oocytes using a two-electrode voltage clamp (Warner model OC725-B, Hamden, CT) with agar-bridged bath electrodes (25). Data were filtered at 8 Hz (~3 dB Bessel filter), digitized at 20 Hz, and analyzed with our custom programs utilizing LabView software (National Instruments, Austin, TX). Current-voltage relations were generated using voltage steps or ramps (56 mVsteps, 1.8 mV/point) between EK or EMG and −30 mV from a given holding potential (usually −60 mV unless noted). Substrate-dependent, current-voltage data were obtained by subtracting control current values measured in the absence of substrate. Leakage I-V data that represented CAATCH1-associated leakage currents exposed by a substrate, were computed by digital subtraction of current in the presence of leakage-exposing substrate from control current (I leakage − I control). Transport-associated I-V data of CAATCH1 currents appearing only in the presence of a solute substrate, computed by the inverse operation (I substrate − I control).

Results—A single clone was obtained with a full-length sequence of 2858 base pairs (CAATCH1; GenBank™ accession no. AF013963). It contains an open reading frame of 1899 base pairs that encodes a polypeptide with 633 amino acid residues (Fig. 1) and has an unglycosylated relative molecular mass of 69,934 with pI = 7.37. A 5'-UTR of 100 bases and a polyadenylated 3'-UTR of 859 bases flank the open reading frame. The predicted topology of CAATCH1 (27, 28) includes 12 putative transmembrane domains, with cytosolic N- and C-terminal segments rich in proline, as well as acidic and basic amino residues (Fig. 1A). Seven consensus phosphorylation sites are located within cytoplasmic loops, and two N-linked glycosylation sites exist on the extracellular loop between the third and fourth membrane-spanning segments. Scanning the GenBank™ data base reveals that the sequence of CAATCH1 is related to Na+/Cl−-dependent solute transporters (e.g. human dopamine transporter hDAT; Fig. 1B) in species ranging from Caenorhabditis elegans to Homo sapiens with 35–45% nucleotide sequence identity and 35–39% amino acid sequence identity (50% similar); such transporters mediate the reuptake of dopamine, serotonin, norpinephrine, GABA, glycine, and proline (8–10, 12, 23, 29–32). The most closely related clone is the M. sexta KAAT1 neutral amino acid transporter (18) (Fig. 1) with overall 92% nucleotide identity and 90% amino acid identity (94% similarity) with CAATCH1. Amino acid differences are scattered throughout the sequence; however, conspicuous sequence differences are found within or near predicted transmembrane domains 6, 11, and 12 as well as in their adjacent hydrophilic, cytosolic C- and N-terminal regions. For example, between CAATCH1 residues Leu906 and Ile977, 25 amino acids (or 31%) diverge, often with nonconservative differences. Moreover, as the results below indicate, KAAT1 and CAATCH1 exhibit striking differences in physiology and function. Parallel experiments conducted on the wild types and several site-directed mutants of both KAAT1 and CAATCH1 cDNAs confirmed that they are functionally distinct transporters.

The functional characteristics of CAATCH1 expressed in Xenopus oocytes were unexpected and collectively did not conform to any known transport system, including KAAT1 or the 

2 B. R. Stevens, Z. Liu, D. H. Feldman, M. A. Hediger, and W. R. Harvey, unpublished observations.
excitable tissue transporters (18, 23, 33–35). Nutrient amino
acid substrates for CAATCH1 elicited three categories of elec-
trophysiological responses (Figs. 2–4): 1) net inward currents,
typified by proline; 2) apparent net “outward” responses typi-
fied by methionine; and 3) a mixed net effect of elicited inward
current plus apparent “outward” current, typified by threonine.

Fig. 1. A, predicted amino acid sequence secondary structure (27, 28) of CAATCH1. Phosphorylation sites are numbered, and the two N-linked glycosylation sites are shown on the large extracellular loop. B, amino acid sequence of CAATCH1 aligned with KAAT1 (18) and hDAT (10). Putative transmembrane regions (27, 28) are overscored with numbered bars. #, N-linked glycosylation sites; *, PKC phosphorylation sites; ‡, protein kinase A/cGMP sites.
Furthermore, the amino acid-elicited responses were modulated by Na\(^+\), K\(^+\), pH, and the transmembrane voltage but not by Cl\(^-\).

The first type of substrate, such as L-proline and its analogue L-pipecolate, elicited the greatest net inward currents of any test substrates in alkaline Na\(^+\) medium (Fig. 2, A and C). These currents were voltage-dependent over the range from \(-150\) to \(+30\) mV (Fig. 3A).

The second type of substrate, such as methionine or leucine, blocked the CAATCH1-associated leakage current, thereby giving apparent “outwardly directed” current traces in Na\(^+\) medium (Fig. 2, A and C). The blockage of a CAATCH1-associated leakage current by these amino acids was characterized by a reduced slope of the current-voltage relation in the presence of substrate, compared with the control conditions. The CAATCH1 leakage current I-V relationship for methionine (Fig. 3B) was obtained by subtracting I-V traces measured in the presence of this leakage-blocking substrate from those measured in its absence. Sonders et al. (10) used this method to reveal the cocaine blockage of the hDAT leakage current, which was independent of cocaine's competitive inhibition of dopamine carrier-mediated uptake via hDAT. Methionine-blocked CAATCH1 leakage currents in Na\(^+\) medium (Fig. 2, A and C) gave an outwardly rectifying I-V relationship \((I_{\text{CON}} - I_{\text{MET}})\) with reversal potentials occurring between \(-10\) and \(-25\) mV (Fig. 3B).

The third type of amino acid, exemplified by threonine, elicited no current or small inward currents (Fig. 2, A and C), yielding an inverted U-shaped (convex) I-V relationship (Fig. 3A), since their CAATCH1-associated inward currents were balanced by their blockage of the CAATCH1-associated leakage current in Na\(^+\) medium (Fig. 2, A and C).

Complete replacement of extracellular Na\(^+\) with K\(^+\) changed the apparent substrate selectivity; thus, threonine (Fig. 2, B and D) elicited larger voltage-dependent inwardly rectifying currents than proline (Fig. 2, B and D; Fig. 3C). Furthermore, amino acids that blocked the leakage current in Na\(^+\) medium (Fig. 2C) generated net inward currents in K\(^+\) medium (Fig. 2D).
CAATCH1 constitutive leakage current inhibited in the presence of the Na\(^+\) medium, whereas it blocked the leakage current in Na\(^+\) medium. The threonine-elicited CAATCH1 current was slightly greater than the concomitant block of the constitutive CAATCH1 leakage current, yielding a convex shaped I-V relationship due to the additive effects of the inwardly rectifying transport-associated current with the inverted outwardly rectifying leakage current. This behavior led to the complex I-V relationship of the difference IV relations increased as a function of Na\(^+\) medium. In K\(^+\) medium, the kinetics of proline- and threonine-elicited proline-activated inward current was fitted to the Michaelis-Menten equation, yielding hyperbolic kinetics that indicated single-site carrier behavior (data not shown). For the Na\(^+\) - activated proline current, \(K_m = 330 \pm 15 \mu M\), whereas for the Na\(^+\) - activated threonine current, \(K_m = 35 \pm 4 \mu M\). For the K\(^+\) - activated proline current, \(K_m = 1900 \pm 270 \mu M\), and for the K\(^+\) - activated threonine current, \(K_m = 235 \pm 5 \mu M\).

Current-voltage relationships were determined for threonine currents activated at increasing concentrations of K\(^+\) and for proline currents activated by increasing concentrations of Na\(^+\). Activation data were then fitted to the Hill equation by non-linear regression, as illustrated in Fig. 5A. The apparent Hill coefficient, \(n_H\), was \(\sim 2\) for both K\(^+\) and Na\(^+\), implying an activation coupling ratio of 2 K\(^+\):1 threonine and 2 Na\(^+\):1 proline.

CAATCH1 does not require any Cl\(^-\) for amino acid-elicited inward current activity (Fig. 5B). Complete replacement of chloride with gluconate in Na\(^+\)-containing medium elicited proline-associated currents that were not significantly different (\(p > 0.05\)) at 100 mM Cl\(^-\) (100 ± 6%) from currents at 0 mM Cl\(^-\) (92 ± 9%). This phenomenon contrasts with the requirement for extracellular CI\(^-\) ion observed in cation-activated monoamine cotransporters and in KAAT1 (8–10, 12, 13, 18, 23, 29, 35, 37). Although unaffected by Cl\(^-\), cation-activated amino acid-elicited CAATCH1 inward currents were activated as a function of pH (or OH\(^-\) concentration) (Fig. 5C). In CAATCH1-expressing oocytes the maximal pH was 9.5 (Fig. 5C), mirroring the in vivo alkalinity of M. sexta midgut (38). The slopes (data not shown) of the difference IV relations increased as a function of pH, in accordance with the increased transport-associated current measured at the fixed holding voltage of ~60 mV (Fig. 5C).

l-Proline in Na\(^+\) medium elicited an inward current that was accompanied by an abrupt acid shift in the unstirred layer surrounding the CAATCH1-expressing oocytes (Fig. 6). Subsequent removal of l-proline abruptly shifted the pH back in the alkaline direction along with arrest of the inward current. The pH shift represented either OH\(^-\) influx or H\(^+\) efflux occurring concurrently with Na\(^+\)-dependent proline-elicited inward current. This effect of CAATCH1 activity on pH contrasts with the

**Fig. 3.** Changes in amino acid-dependent current-voltage relations as a function of Na\(^+\) or K\(^+\). All recordings were made from the same representative cell. Voltage ramps (+30 to ~150 mV during 5 s) were applied during constant superfusion of each amino acid (500 \(\mu M\)) in either Na\(^+\) or K\(^+\) medium. A, proline- or threonine-elicited currents in Na\(^+\) medium. The threonine-elicited CAATCH1 current was slightly greater than the concomitant block of the constitutive CAATCH1 leakage current, yielding a convex shaped I-V relationship due to the additive effects of the inwardly rectifying transport-associated current with the inverted outwardly rectifying leakage current. B, leakage block I-V curve elicited by methionine in Na\(^+\). Values represent CAATCH1 constitutive leakage current inhibited in the presence of the amino acid; the CAATCH1 reversal potential of this particular example was ~15 mV. C, proline- or threonine-elicited currents in K\(^+\) medium.
CAATCH1 current-voltage relations with proline and methionine combinations marked by reversal potentials as a function of pH (data not shown) gave a m500 energized by pH gradients or co-activated by H1. pH relationships of other eukaryotic transporters, which are CAATCH1 currents in Na+ medium. Proline alone (P; 500 μM) elicited an inward transport-associated current, whereas methionine alone (M; 500 μM) blocked the constitutive CAATCH1 leakage current. The proline-elicited steady-state inward current was increasingly inhibited by 0.005–500 μM methionine. Blanked voltage ramp recordings are marked by triangles. Membrane voltage was clamped at −60 mV. B, CAATCH1 current-voltage relations with proline and methionine combinations in Na+ medium. I-V relations were derived from voltage ramp commands as in A. For clarity, a limited number of proline and/or methionine I-V curves are shown. For each steady-state I-V curve except Met only (leak), the data were plotted according to the convention for transport-associated currents, i.e. corrected for base line by digital subtraction of control base-line ramp currents (measured immediately prior to the application of amino acids) from the ramp current measured during the amino acid application. Met only (leak) shows the leakage I-V relation (dashed line) in its conventional form, in which current in the presence of methionine was subtracted from the base-line control current. Met only (inverse leak) represents the data (dotted line) plotted in the form normally used for transport-associated currents. C, methionine inhibition of inward proline-induced CAATCH1 current. To isolate net transport-associated currents (at leakage current = 0 nA), measurements were obtained from the current records during the voltage ramps (see A and B) at a voltage representing the reversal potential of the leakage current (i.e. −24 mV in this example). These data represent methionine inhibition of only the proline-associated net current in Na+ medium.

pH relationships of other eukaryotic transporters, which are energized by pH gradients or co-activated by H+ inward flux (36).

Current-voltage relationships were measured at six extracellular pH values from pH 7.0 to 9.4 in the absence of external monovalent cations (replacement by NMG−). A linear fit of the reversal potentials as a function of pH (data not shown) gave a slope of −11 ± 0.2 mV/pH unit. This value is less than that predicted for a perfect Nernst potential for H+ (or OH−), indicating that other ions (probably intracellular K+ or Na+) carry a greater proportion of the current through the leakage pathway.

CAATCH1 cation-associated current-voltage relationships, independent of the substrate-associated currents, were observed in the absence of leakage blocking amino acids (Fig. 7A). Here, the rightward shift in the reversal potential and the positive slopes indicates a conductance for several cations, with a preference of Li+ > Na+ > K+. However, when methionine was present (Fig. 7B), the negative slope (inverse leakage current) of the difference I-V relationship in Na+ unmasked the CAATCH1 leakage current that is apparently constitutively present in the absence of any test amino acid. The inwardly rectifying positive slopes of the difference curves in Li+ and K+ (Fig. 7B) indicate that these cations can also activate methionine-associated carrier currents.

In addition to its rheogenic properties, CAATCH1 also catalyzed carrier-mediated uptake of H-labeled amino acid substrates (25 μM). In intact oocytes expressing CAATCH1, uptake values obtained in medium with Na+ as the only alkali cation, under non-voltage-clamped conditions and corrected for non-specific uptake measured in water-injected oocytes, were as
values in the Na\(^+\)-dependent proline-elicited inward current and pH of extracellular unstirred layer. A, inward CAATCH1 current elicited by 500 \(\mu\)M proline in Na\(^+\)-free K\(^+\) medium. L-Proline in Na\(^+\) medium (pH 8.410 \pm 0.003) was slowly superfused by a peristaltic pump. A micro-pH electrode was positioned in the extracellular medium 50 \(\mu\)m from the surface of a CAATCH1-expressing oocyte that was impaled with a two-electrode voltage clamp. Current and extracellular pH measurements were obtained simultaneously. Gaps appear where deflections resulting from applied voltage ramps were removed. B, acute pH shifts in extracellular medium were coincident with Na\(^+\)/proline-elicited inward current. An abrupt alkaline pH shift occurred upon removal of proline, coincident with cessation of Na\(^+\)/proline-elicited inward current.

follows (pmol/min/oocyte; mean \pm S.E.): L-proline, 2.10 \pm 0.20; L-threonine, 1.98 \pm 0.30; L-alanine, 0.82 \pm 0.30; and L-methionine, 0.2 \pm 0.10. Repeated attempts to measure uptake in medium with K\(^+\) as the only alkali cation varied quite widely from batch to batch of injected oocytes, yielding error values often about \(\pm 100\%\). However, radiolabeled amino acid uptake values in the Na\(^+\)-free K\(^+\) medium were generally greater than water-injected control oocytes but less than uptake in K\(^+\)-free Na\(^+\) medium. The inability to derive meaningful measurements under Na\(^+\)-free K\(^+\) experimental conditions is probably due to a lack of significant driving force for transport (i.e. \([K^+]_{out} \sim [K^+]_{in}\)).

**DISCUSSION**

CAATCH1 is a unique clone that shares some structural and functional characteristics with Na\(^+\),Cl\(^-\) -coupled neurotransmitter transporters of excitable tissue but also shares characteristics of epithelial nutrient amino acid transport systems. The unanticipated characteristics of CAATCH1 included Cl\(^-\) insensitivity, inward currents that are elicited by nutrient amino acids and activated by either K\(^+\) or Na\(^+\), substrate preference that depends on the activator cation (e.g. threonine with K\(^+\) but proline with Na\(^+\)), alkaline pH activation increasing to pH 9.5, voltage dependence, and, notably, the inverted U-shaped current-voltage relationship of a ligand-inhibited "apparently outward" leakage current initially ascribed to neurotransmitter transporters such as DAT (8–10). The DAT, NET, SERT, GABA, GAT1, EAATx, GLAST, GLT, PROT, and GlyTx transporters each mediate uptake of solute (primarily neuroactive amines) via a carrier function, yet they also exhibit substrate-independent ion leakage channels that can be inhibited by pharmacological agents in some instances (1–5, 8–13, 29, 35–37, 40–43). Na\(^+\)-dependent leakage currents have also been reported in nonexcitable tissues (e.g. a thyroid Na\(^+\)/I\(^-\) uptake system (15), myo-inositol (16), and Na\(^+\)/glucose (SGLT1) transporters (3, 17) and ASCT-x Na\(^+\), Cl\(^-\) dependent transporters broadly selective for both neutral and acid amino acids (2, 5)). The mammalian amine and amino acid transporters are strictly dependent on Cl\(^-\), unlike CAATCH1 (Fig. 5). Related insect transporters, dSERT of Drosophila (48) and a Manduca GABA transporter (30), each retain about 50% of Na\(^+\)-dependent serotonin or GABA uptake when all Cl\(^-\) is replaced by gluconate.

KAAT1 and CAATCH1 appear to be related, but they are different proteins with different functions. Although they are 90% identical, they diverge at 62 positions distributed among many sites within the overall structure (Fig. 1), as described under "Results." It is possible to conceive of complex patterns of alternate splicing of transcripts derived from a single gene to account for these differences, although RNA editing of a single transcript or a gene duplication could also account for the variations. Both clones display the property of voltage-dependent, substrate-elicited currents activated by K\(^+\) at alkaline pH (Figs. 2, 3, and 5) (18), and they both display currents elicited from batch to batch of injected oocytes, yielding error values...
by Na⁺ in the absence of substrates (Fig. 7) (39), but the substrate selectivity and electrophysiological properties of the two transporters are otherwise quite different. For example, in addition to the list of CAATCH1’s unique properties summarized above, the CAATCH1 leakage current is modulated by micromolar methionine (Figs. 2–4), whereas a KAAT1 constitutive current is not inhibited by any known agent (34, 39). Moreover, our initial studies of single site and double site mutations show striking functional differences between CAATCH1 and KAAT1.²

With an apparent reversal potential of approximately −20 mV in Na⁺ medium, the observed CAATCH1 leakage phenomenon is probably a complex event, with the actual ion(s) responsible for it being unknown at present. The CAATCH1 conductance as well as its net inward holding currents measured in the presence of various single alkali cation species are greater than values obtained when NMG⁺ replaces all alkali cations (Fig. 7). This suggests that the CAATCH1 leakage pathway could be conductive to at least Li⁺, K⁺, and Na⁺. The presence of a high Li⁺ conductance is consistent with observations for other members of the Na⁺-dependent neurotransmitter transporter family. Furthermore, in alkali cation-free NMG⁺ medium, CAATCH1 holding currents increased as a function of extracellular [H⁺], with the J-V slopes increasing at lower pH (giving reversal potentials at −11 mVpH unit), suggesting that protons may also permeate the leakage pathway (see “Results”). The lack of Cl⁻ activation and the dependence on alkaline pH (Figs. 5 and 6) raises the possibility that OH⁻ may serve as an activator anion for CAATCH1. It is difficult to study the ion dependence of the ligand-inhibitable leakage pathway in CAATCH1 because it is detectable only in the presence of Na⁺ (Fig. 2, C and D). Taken together, our observations suggest that H⁺ influx and/or OH⁻ efflux may contribute to the net leakage current in the absence of Na⁺ but that Na⁺ is the major contributor when it is present.

The current-voltage, pH, substrate concentration, and ion concentration data suggest that CAATCH1 may exist in one or more states, as has been postulated for amine or amino acid neurotransmitter transporters (3–5, 8). At least two states could exist that are regulated by the available cation and amino acid substrates: 1) a Na⁺-dependent state having a constitutive leakage pathway that is inhibited by certain amino acids, such as methionine, but that can generate inward transport-associated currents in the presence of a narrow spectrum of amino acids (e.g., proline) (some amino acids, such as threonine, may both inhibit the leakage current and produce transport-associated currents) and 2) a K⁺-dependent state in which transport-associated currents are elicited by a broad spectrum of neutral amino acids, such as threonine or methionine, whose predominant effects in the presence of Na⁺ are the inhibition of the leakage currents.

The leakage current and its modulation by amino acid ligands are not predicted by traditional models but are in accord with recent observations in other transporters (13, 14, 40, 44–46). Our data are consistent with a model in which 1) the test amino acids bind to a common receptor site on CAATCH1; 2) the avid binding of most test amino acids (except proline) prevents inward leakage current in Na⁺ medium, where the predominant effect of methionine is to expose the CAATCH1-associated leakage conductance; and 3) the binding affinity for most amino acids is reduced in K⁺ medium, thereby allowing the transport cycle to be completed with concomitant generation of inward carrier-associated currents.

CAATCH1 properties can account for several characteristics of amino acid transport observed in insect larval epithelium (38). The characteristics of CAATCH1-dependent radiolabeled amino acid uptake by oocytes (see “Results”) are consistent with those found in radiotracer uptake studies in isolated membrane vesicles from Manduca midgut (38). This earlier vesicle work demonstrated multiple, physiologically separate uptake “systems” for alkali cation-activated amino acid co-transport in Manduca epithelial cells (47). The K⁺-activated, leucine-prefering KAAT1 transporter was also previously expression-cloned from Manduca RNA (18).

In conclusion, the amino acid carrier and apparently ion leakage properties of CAATCH1, previously attributed to the organic solute transporters of excitable tissues, provide insights into the means of electrogenic amino acid uptake in the highly alkaline (pH > 10) caterpillar midgut. The alkaline is maintained by an H⁺-V-ATPase that generates an approximately −240-mV transmembrane voltage in conjunction with a putative K⁺/2H⁺ antiporter (38). The alkaline milieu breaks down dietary tannins that block amino acid absorption, while vesicle studies show that the main function of the large voltage is to drive K⁺ and amino acids into the epithelial cells via transport systems (38). The characteristics of CAATCH1 described here pave the way for a detailed electrophysiological analysis of CAATCH1’s transport solute/current stoichiometry and pre-steady state kinetics.

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