Amino Acid Transporter CAATCH1 Is Also an Amino Acid-gated Cation Channel*

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CAATCH1 (cation-amino acid transporter/channel) is a recently cloned insect epithelial membrane protein related to mammalian Na⁺-, Cl⁻-coupled neurotransmitter transporters (Feldman, D. H., Harvey, W. R., and Stevens, B. R. (2000) J. Biol. Chem. 275, 24518–24526). In the present study we analyze the relationship between CAATCH1-mediated amino acid transport and ion fluxes by utilizing the Xenopus oocyte expression system in conjunction with electrophysiology and radiotracer uptake. Simultaneous flux measurements reveal that electrical currents and amino acid transport are thermodynamically uncoupled. This observation is supported by measuring significant uptake even in the absence of external alkali cations. Remarkably, CAATCH1-associated Na⁺ or K⁺ currents are large and do not saturate with voltage nor with cation concentration. These currents reverse in Nernstian fashion, thereby conferring channel activity to CAATCH1. Upon step-changes in the membrane potential, CAATCH1-expressing oocytes exhibit transient currents. Detailed analyses of these transients in the absence and presence of amino acids reveal direct ligand-protein interaction, demonstrating that binding by different amino acids (e.g. proline, threonine, methionine) differentially affects the state probability of CAATCH1 but has no effect on the maximal charge movement (Qmax). Together these data suggest that CAATCH1 is a multifunction membrane protein that mediate thermodynamically uncoupled amino acid uptake but functions predominantly as an amino acid-gated alkali cation channel.

CAATCH1 (cation-amino acid transporter/channel) is a 70-kDa membrane protein recently cloned from Manduca sexta midgut (1). Although CAATCH1 was isolated from a larval nutrient absorptive epithelium, its sequence and electrophysiological properties are similar to mammalian Na⁺-, Cl⁻-coupled cotransporters (2) whose members utilize electrochemical Na⁺- and amino acid gradients to drive the re-uptake of neurotransmitters such as dopamine (3), serotonin (4), glutamate (5), and γ-aminobutyric acid (6). The greatest sequence identity (80%) is found with the M. sexta K⁺-coupled amino acid transporter KAAT1 (7).

Like KAAT1, CAATCH1 mediates amino acid uptake, and exhibits both an amino acid-elicited current and an alkali cation current in the absence of amino acids (1). However, the two proteins differ markedly in their amino acid substrate specificity and electrophysiological profiles. Notably, the CAATCH1 Na⁺ current is inhibited by specific amino acids, reminiscent of the ligand (e.g. cocaine)-modulated current in the human dopamine transporter (3). This led us previously to suggest that CAATCH1 may be involved in the regulation of Na⁺ fluxes in the larvae and thus may play a broader role than that of a “classical” cation-coupled nutrient amino acid cotransporter (1).

The goal of this work, therefore, was to study the relationship between the transport of representative amino acids (L-proline, L-threonine, and L-methionine) and fluxes of cations (Na⁺ and K⁺). To this end we have analyzed CAATCH1 expressed in Xenopus oocytes by using tracer flux and the two-electrode voltage-clamp technique. Remarkably, amino acid uptake by CAATCH1 is thermodynamically uncoupled from ion fluxes. However, the most intriguing finding of the present report is that CAATCH1 functions predominantly as an amino acid-modulated alkali cation channel.

EXPERIMENTAL PROCEDURES

CAATCH1 Expression, Electrophysiology, and Uptake Assays—CAATCH1 was expressed in Xenopus laevis oocytes and analyzed using the two-electrode voltage-clamp method, as described previously (1, 8). For transport and electrophysiological experiments, oocytes were bathed in an assay buffer composed of 1 mM MgCl₂, 1 mM CaCl₂, 10 mM TAPS-NMG⁺, pH 8.0, and a combination of Na⁺ or K⁺ chloride salts with NMG⁺ to give a final concentration of 100 mM. Uptake of 500 μM of H-labeled L-amino acids (Amersham Pharmacia Biotech) was performed in non-clamped oocytes or was monitored under voltage clamp for 20 min (8).

Data Analysis—Amino acid-evoked currents were obtained as the difference between currents in the presence and absence of amino acid. For evaluation of transient currents, total currents were fitted to the following equation (1),

\[ I_{\text{total}}(t) = I_e^{(e^{-t/\tau_2})} + I_c^{(e^{-t/\tau_1})} + I_{\text{ss}} \]  

where \( I_{\text{total}} \) is the total current, \( I_e \) and \( I_c \) are membrane capacitive current, transient current, and steady-state current, respectively, with \( \tau_e \) and \( \tau_c \) as the time constant of \( I_e \) and \( I_c \); \( I_{\text{ss}} \) represents the membrane potential at which 50% of the total charge has moved in the membrane electric field, \( z \) is the apparent valence of the moveable charge, and \( F \) and \( R \) are the usual units. Data were fitted using non-linear regression algorithms of SigmaPlot (version 6.0, SPSS Inc., Chicago, IL). Unless noted

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RESULTS

CAATCH1 Steady-state Na\(^+\) and K\(^+\) Currents—Steady-state current responses to a series of 100-ms voltage steps (−150 mV to +50 mV) were measured at [Na\(^+\)]\(_{\text{ex}}\) or [K\(^+\)]\(_{\text{ex}}\) varying from 0 to 100 mM. Fig. 1A shows representative current-voltage (I–V) relationships for a CAATCH1-expressing oocyte or an non-injected control oocyte in the absence of external amino acid. For CAATCH1-expressing oocytes, the current magnitude increased with increasing [cation]\(_{\text{ex}}\) at negative potentials, but was much less affected at potentials more positive than +10 mV. Each of the currents reversed but did not become saturated at either the highest cation concentration nor the largest hyperpolarizing voltage (−150 mV). Although currents in the absence of external Na\(^+\) or K\(^+\) (i.e. in the presence of NMG\(_{100}\)) were similar for CAATCH1-expressing oocytes and control oocytes at −150 mV (−75 nA), the outward currents for CAATCH1-expressing oocytes greatly exceeded those observed in control oocytes. The reversal potential of currents in NMG\(_{100}\) was −95 mV for CAATCH1-expressing oocytes and −35 mV for control oocytes. Increasing [Na\(^+\)]\(_{\text{ex}}\) to 100 mM had no significant effect on the current magnitude or the reversal potential in control oocytes. However, raising [K\(^+\)]\(_{\text{ex}}\) to 100 mM increased the current magnitude in control oocytes at potentials more negative than −10 mV (−260 nA at −150 mV) and shifted the reversal potential toward more positive potentials (−15 mV at K\(_{100}\)). On the other hand, for CAATCH1-expressing oocytes the reversal potential of the currents was shifted by 58 ± 2 mV and 59 ± 2 mV per 10-fold change in internal Na\(^+\) and K\(^+\) concentration, respectively (Fig. 1B).

To obtain the net cation-induced currents, the currents observed in NMG\(_{100}\) were subtracted from those in the presence of various concentrations of [Na\(^+\)]\(_{\text{ex}}\) and [K\(^+\)]\(_{\text{ex}}\). The resulting currents were slightly decreased in their magnitude at hyperpolarizing potentials. However, the outward rectification at depolarizing potentials observed only for CAATCH1-injected oocytes, which is due to K\(^+\) outward conduc-

ductance, was eliminated by this method, and thus the currents became saturated with depolarization (data not shown).

Addition of 0.5 mM L-proline, L-threonine, or L-methionine to the presence of 500 μM L-proline (□), L-threonine (△), or L-methionine (○) in 100 mM Na\(^+\)]\(_{\text{ex}}\) and [K\(^+\)]\(_{\text{ex}}\). B, net t-methionine- or t-threonine-induced currents in 1 mM (●), 25 mM (△), and 100 mM (□) Na\(^+\)]\(_{\text{ex}}\). Data shown in Figs. 1A, 1B, 2A, and 2B are from the same oocyte.

Fig. 1. Voltage dependence of steady-state currents. A, currents of a representative oocyte 5 days after injection with CAATCH1 cRNA in the presence of NMG\(_{100}\) or various [Na\(^+\)]\(_{\text{ex}}\) or [K\(^+\)]\(_{\text{ex}}\) in the absence of amino acid are plotted as function of the membrane potential (V\(_{m}\)). Symbols indicate 0 mM (○) (left panel only), 10 mM (●), 25 mM (△), and 100 mM (□) [Na\(^+\)]\(_{\text{ex}}\) or [K\(^+\)]\(_{\text{ex}}\). For comparison, currents in the absence of external Na\(^+\) or K\(^+\) (i.e. NMG\(_{100}\)) and in Na\(_{100}\) or K\(_{100}\) are shown for non-injected control oocytes. B, reversal potentials (ER) are shifted by 58 ± 2 mV and 59 ± 2 mV/log changes in [Na\(^+\)]\(_{\text{ex}}\) and [K\(^+\)]\(_{\text{ex}}\), respectively. Data are from oocytes from different batches (n = 3), including those shown in A, and errors represent S.E.
amino acid from the total currents in presence of 0.5 mM amino acid. In K_{100} the amino acid-evoked currents with L-threonine, L-proline, and L-methionine increased with hyperpolarization and were comparable (−1 to −1.8 μA at −150 mV). Increasing [K\(^+\)]\(_{ex}\) from 1 to 100 mM increased the amino acid-elicited inward currents, but these currents did not become saturated at either the most hyperpolarizing potential nor with the highest external [K\(^+\)] (data not shown). In Na\(_{100}\) 0.5 mM L-proline elicited the largest amino acid-induced inward current, which did not become saturated with hyperpolarization (−2.3 μA at −150 mV) whereas the L-threonine-evoked current was much smaller (−0.5 μA at −150 mV). Addition of 0.5 mM L-methionine in Na\(_{100}\) inhibited the magnitude of the total current, which was reflected as an L-methionine-elicited outward current at hyperpolarizing voltages (Fig. 2, A and B) (1). At [Na\(^+\)]\(_{ex}\) > 5 mM L-methionine inhibited the current, and the magnitude of the inhibition increased by increasing [Na\(^+\)]\(_{ex}\). However, at [Na\(^+\)]\(_{ex}\) ≤ 5 mM addition of L-methionine increased the inward currents at negative potentials (Fig. 2B). Similar to L-methionine, addition of 0.5 mM L-threonine to [Na\(^+\)]\(_{ex}\) from 1 to 100 mM generated inward currents that increased with hyperpolarization. At [Na\(^+\)]\(_{ex}\) ≥ 25 mM the magnitude of the inward currents at −150 mV decreased, but the inhibition became more distinct between −10 and −130 mV, yielding complex (convex shaped) V-I relations. In the absence of external alkali cation (NMG\(^+\)\(_{100}\)), addition of either amino acid to the bathing solution generated only marginal voltage-independent amino acid-evoked inward currents of about 10 nA (data not shown). No amino acid-elicited currents were detectable in control oocytes by varying [Na\(^+\)]\(_{ex}\) and [K\(^+\)]\(_{ex}\) from 1 to 100 mM (data not shown).

Amino Acid Uptake—Next, we assayed 500 μM \(^{3}\)H-labeled L-proline, L-threonine, and L-methionine uptakes under non-clamped conditions in oocytes expressing CAATCH1 and in control oocytes (Fig. 3A). In the same batch of oocytes used for these uptake studies, CAATCH1-expressing oocytes exhibited a membrane potential of −51 ± 5 mV (n = 5), and for non-injected oocytes −36 ± 2 mV (n = 3). The greatest uptake rate was observed with L-proline in Na\(_{100}\). However, unlike L-proline, which increased the magnitude of the inward currents, significant uptake was observed for L-threonine and L-methionine, even though these amino acids inhibited (L-methionine) or slightly increased (L-threonine) the currents at a comparable test potential under voltage-clamped conditions (−30 mV, see Fig. 2, A and B). Uptake in K\(_{100}\) was less than in Na\(_{100}\), but was similar among all three amino acids. In the absence of external cations (NMG\(_{100}\), Fig. 3A, inset) or 1 mM [Na\(^+\)]\(_{ex}\) (data not shown), oocytes expressing CAATCH1 exhibited about 80% L-methionine uptake compared with Na\(_{100}\). The background uptake activity in control oocytes was similar among all three amino acids (about 25 pmol of amino acid × oocyte\(^{-1}\) × 20 min\(^{-1}\)).

To provide direct comparison between amino acid flux and electrical currents in the same oocyte, uptake was also performed under voltage-clamped conditions. Addition of 500 μM \(^{3}\)H-labeled L-proline, L-threonine, or L-methionine to K\(_{100}\) bathing solution reversibly increased the inward current (L-methionine is shown in Fig. 3B). Integration of the inward current with time revealed the net amino acid-evoked charge movement. In the same oocyte the \(^{3}\)H content was also determined. Fig. 3C shows the molar ratio of amino acid uptake to charge movement as function of \(^{3}\)H amino acid uptake for each [cation]100/amino acid combination. In K\(_{100}\) the molar ratios of inward movement of positive charges and L-proline, L-threonine, and L-methionine uptake varied from 0.6 to 2.4 among individual oocytes, a variation greatly exceeding the exper-
and uptake did not exceed 25% of the CAATCH1-expressing oocytes (Fig. 3B).

**Transient Currents in CAATCH1**—In Na\(^+\), step-changes in the membrane potential induced transient currents in CAATCH1-expressing oocytes (Fig. 4A) but not in non-injected oocytes (Fig. 4C), thus reflecting CAATCH1-associated charge transfer in the membrane dielectric field. Fig. 4A (upper panel) shows current responses in the absence of amino acid in 25 mM [Na\(^+\)]\(_{ex}\) For each voltage pulse, currents in the ON and OFF response were fitted to Equation 1. After a fast capacitive component with a time constant \(\tau = 1\) ms, which was independent of the membrane potential, each current relaxed to a steady state with a single time constant. The time constants in the ON and OFF response were voltage-dependent and exhibited a Gaussian distribution with maximum \(\tau_{ON} = \tau_{OFF} \sim 5.9\) ms at about \(-27\) mV (data not shown). Subtracting the capacitive and steady-state component yielded the CAATCH1-associated transient currents. These transients were equal and opposite in sign in the ON and OFF response (the ON response is shown in Fig. 4B, upper panel). To obtain the charge transfer, the current transients were integrated with time for each voltage and plotted as a function of the membrane potential. The data were described by the Boltzmann equation (see Equation 2) with \(Q_{\text{max}} = 20\) nC \(\pm 5\), \(V_{0.5} = -47\) \(\pm 1\) mV, and \(z = 1.0 \pm 0.03\) (Fig. 4, D and E). Addition of 0.5 mM \(\alpha\)-threonine (Fig. 4A, lower panel; or \(\alpha\)-methionine, data not shown) dramatically affected the current transients shifting \(V_{0.5}\) to \(-0.5 \pm 2\) mV, but with no effect on \(Q_{\text{max}}\) (21 nC \(\pm 2\)) or \(z\) (1 \(\pm 0.02\)). Although the relaxation time constants at potentials \(\leq -70\) mV were indistinguishable from the capacitive time constants, the greatest relaxation time constant \((\tau_{ON} = \tau_{OFF} \sim 14\) ms) was detected at \(+50\) mV. At 25 mM [Na\(^+\)]\(_{ex}\) addition of \(\alpha\)-proline had only a minor effect on the transient currents. However, increasing [Na\(^+\)]\(_{ex}\) \(\geq 50\) mM slightly shifted \(V_{0.5}\) toward more positive potentials (Fig. 4E).

In K\(^+\), the relaxation currents observed for CAATCH1-expressing oocytes were much smaller than those in Na\(^+\) (data not shown). Because the magnitude and the relaxation time constants of these transitions were similar to those observed in non-injected oocytes (Fig. 4C), we were unable to obtain reliable transient current kinetics for K\(^+\).

Fig. 4D shows a plot of the charge \((Q)\) as a function of voltage for [Na\(^+\)]\(_{ex}\) from 5 to 100 mM in the absence of amino acid. Increasing [Na\(^+\)]\(_{ex}\) shifted the Q–V curves to more positive potentials, accompanied by a positive shift of \(V_{0.5}\) (see Fig. 4E). From 10 to 75 mM [Na\(^+\)]\(_{ex}\) the value of \(Q_{\text{max}}\) was \(20 \pm 2\) nC. At the extreme [Na\(^+\)]\(_{ex}\) (<10 or >75 mM) the Q–V curves did not become saturated at hyperpolarizing or depolarizing potentials, thereby precluding accurate Boltzmann fits outside this range. For all test [Na\(^+\)]\(_{ex}\), \(z \sim 1\).

Increasing [Na\(^+\)]\(_{ex}\) from 10 to 100 mM shifted \(V_{0.5}\) from \(-68 \pm 1\) mV to \(-5 \pm 4\) mV (Fig. 4E). This shift exhibited a slope of \(58 \pm 3.5\) mV per 10-fold change in [Na\(^+\)]\(_{ex}\) in the absence of amino acids. Addition of 0.5 mM \(\alpha\)-proline shifted \(V_{0.5}\) at [Na\(^+\)]\(_{ex}\) \(\geq 50\) mM slightly toward depolarizing potentials. A more dramatic shift of \(V_{0.5}\) toward positive potentials was observed by the 0.5 mM \(\alpha\)-threonine and to a greater extent with \(\alpha\)-methionine at [Na\(^+\)]\(_{ex}\) \(\geq 5\) mM. It is noteworthy that the shift of \(V_{0.5}\) to depolarizing potentials limited adequate Boltzmann equation fitting in these cases. In the absence of external Na\(^+\) or presence of 1 mM [Na\(^+\)]\(_{ex}\) no significant effect on \(V_{0.5}\) was observed.

Fig. 5A shows the positive shift of the Q–V curve along the voltage axis induced by the addition of 2 mM \(\alpha\)-methionine in 10 mM [Na\(^+\)]\(_{ex}\) This shift was not accompanied by changes in either \(Q_{\text{max}}\) or \(z\). The methionine-induced \(V_{0.5}\) shift \((\Delta V_{0.5})\)
saturated with l-methionine with a half-maximal concentration ($K_{0.5}$) of 173 ± 5 μM (Fig. 5B) (13). Similar results were obtained with l-threonine in 25 mM [Na$^+$]$_{in}$ ($K_{0.5}$ = 138 ± 6 μM). At lower [Na$^+$]$_{in}$ (5 mM for l-methionine, or 10 mM for threonine) addition of [amino acid] < 100 μM shifted $V_{0.5}$ toward hyperpolarizing potentials. No shift of $V_{0.5}$ was observed at [amino acid] = 100 μM, but above this [amino acid] $V_{0.5}$ was shifted toward positive potentials.

**DISCUSSION**

In the present study we exploit the Xenopus oocyte expression system in conjunction with electrophysiological and flux measurements to analyze the relationship between CAATCH1-associated amino acid transport and electrical currents. CAATCH1 mediates amino acid uptake uncoupled from electrical ion currents, yet it predominantly behaves as an amino acid-modulated alkali cation channel. These conclusions are based on several lines of evidence. Amino acid uptake and amino acid-elicted electrical currents at $V_m$ ~ −30 mV (see Figs. 2B and 3A) indicate that amino acid transport is not accompanied by stoichiometrically fixed charge movement, even though the highest uptake activity and amino acid-elicted currents were observed for l-proline in the presence of Na$^+$. Strikingly, although l-methionine is transported, it inhibits the magnitude of the currents. This directly contradicts the accepted prerequisites for thermodynamically coupled cotransport (10, 14). In addition, l-methionine flux is also observed in the absence of external alkali cations. In the absence of external Na$^+$ or K$^+$ only marginal inward currents are evoked after addition of either amino acid, and thus these results indicate that (i) CAATCH1-mediated amino acid transport is not necessarily accompanied by ion movement (similar observations are also reported for amino acid uniport in Bombyx mori larval midgut (15)); and (ii) Na$^+$ and K$^+$ represent the charge-carrying ions through CAATCH1.

The latter conclusion is also in line with the high sequence similarity between CAATCH1 and other membrane proteins that are permeable to Na$^+$ and K$^+$ (5, 16, 17). In addition, it is unlikely that the generation of an endogenous non-selective cation channel (18) is induced by the expression of CAATCH1 in oocytes. This is because equimolar replacement of NMG$^+$ with 50 mM Ca$^{2+}$, or lanthanoids (La$^{3+}$, Er$^{3+}$, or Eu$^{3+}$) [chloride salts] did not significantly modify the currents compared with those observed in NMG$^+$ (data not shown).

To provide a direct comparison of amino acid flux and charge movement over the same time course in the same oocyte and to eliminate limitations due to cell-to-cell variability in protein expression, we analyzed tracer uptake under voltage-clamped conditions. In line with the above conclusions, CAATCH1-mediated amino acid/charge flux ratios vary considerably among individual oocytes and between amino acids in Na$^+$ or K$^+$, thus arguing against stoichiometrically fixed cation-coupled amino acid transport as predicted by the determination of the Hill coefficients by cation activation kinetics (1). However, this kinetic parameter represents a qualitative indicator of the apparent binding cooperativity (11, 14, 19, 20). Unlike direct amino acid and ion flux measurements in the same oocyte, the Hill activation coefficient cannot be regarded as a true measure of the coupling stoichiometry (14). Finally, in a coupled system, Na$^+$- and K$^+$-driven amino acid cotransport would have a similar coupling mechanism, i.e. the energy stored in the electrochemical concentration gradient of either coupling cation would be transduced into transport work, as recently shown for Na$^+$- and H$^+$-coupled glucose symport by hSGLT1 (8). Therefore, the present data reveal that the electrogenic properties of CAATCH1 mirror the effect of amino acid on the thermodynamically uncoupled electrical current similar to the effect of pharmacological ligands (e.g. cocaine, dopamine) on the human dopamine transporter-associated currents (3) and the ligand-gated ion channel conductance reported for the Drosophila serotonin transporter (21) (Fig. 6). However, the CAATCH1-associated currents are much greater in magnitude than those observed for these transporters.

The present report emphasizes that CAATCH1-associated electrical currents reflect a channel conductance for alkali cations. This conclusion is based on the fact that CAATCH1 exhibits large Na$^+$ and K$^+$ (and Li$^+$) currents (Fig. 1A). These currents are dependent on both cation concentration and membrane voltage but do not become saturated by either condition. Most importantly, these currents reverse according to the Nernst-Planck relation (Fig. 1B).
age currents have been described previously for several transport proteins (8, 22, 23) and represent unipolar of the coupling cation(s) (24). However, in striking contrast to CAATCH1 these leakage currents exhibit saturation kinetics.

Upon step-changes of the membrane potential, CAATCH1 exhibits transient currents. These transients result from the distribution of charges due to movement of polar residues and/or the movement of the permeating cations within the transmembrane electrical field. CAATCH1 transient and steady-state currents are intimately associated, thus reflecting the correlation between conductance and state probability of the channel. This conclusion is supported by the following observations: (i) transient currents in Na⁺ are only observed in oocytes expressing CAATCH1; (ii) charge transfer (Q) becomes saturated with either hyperpolarization or depolarization and is (iii) equal and opposite in sign in the ON and OFF responses, (iv) the maximal charge transfer (Qmax) is (v) a function of [Na⁺]ex and gives a 58 ± 3.5 mV shift per 10-fold change in [Na⁺]ex, indicating binding of a single Na⁺ as predicted from steady-state current analysis (Hill coefficient = 1); and (vii) V0.5 but not Qmax nor z is affected by amino acids. The latter observation is most apparent for amino acids that inhibit the steady-state currents, strongly suggesting that direct interaction of the amino acid with CAATCH1 affects the state probability. Even though proline, threonine, and methionine are each indeed transported, they do not participate in a cation-coupled cotransport mechanism. Unlike cotransported substrates in a "prototypical" cotransporter (e.g. glucose via SGLT1) (6, 8, 9, 13), these amino acids do not affect the maximal charge (Qmax) in CAATCH1.

Detailed analyses of the effect of amino acids on the V0.5 shift show saturation kinetics, reflecting high affinity amino acid binding at specific [Na⁺]ex (Fig. 5). Because the direction of the current relaxations observed in control oocytes in K⁺ may be hidden by the current relaxations observed in control oocytes in K⁺ (Fig. 4C). Alternatively, as observed for the transients in the presence of t-threonine, which were indistinguishable from the capacitive relaxations at hyperpolarizing potentials, the charge distribution in presence of K⁺ may simply be too fast to be resolved (<1 ms). It is shown for KAAT1 that K⁺-dependent charge transfer is only detectable at high hyperpolarizing potentials using a different pulse protocol (16, 17, 31). However, the instability of CAATCH1-expressing oocytes at potentials more negative than −150 mV precluded us from applying a similar method. To overcome these problems and gain precise control of the experimental conditions, the application of refined methods such as the cut-open oocyte technique (32) would help to further analyze CAATCH1 in more detail.

Taken together, the data of the present report emphasize that CAATCH1, upon expression in *Xenopus* oocytes, represents a dual function protein that mediates amino acid transport but predominantly functions as an amino acid-gated alkali cation channel. Based on these findings CAATCH1 may be a new prototype of non-selective, highly regulated alkali cation translocation systems. Inasmuch as Na⁺ is essential for cell functions in other tissues of insects (33, 34) that operate by a classical Hodgkin-Huxley mechanism, i.e. nerve and muscle cells, it is plausible that CAATCH1 may play a major role in *M. sexta* cation homeostasis.

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**Amino Acid-gated Cation Channel CAATCH1**
