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The Complete Rate Equation, Including the Explicit Dependence on Na^+ Ions, for the Influx of α-Aminoisobutyric Acid into Mouse Brain Slices

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Summary. The rate equation, including dependence on Na^+-ion concentration for the influx of α-amino-*iso*butyric acid into mouse brain slices incubated in isotonic glucose medium at 37 °C, is $v=0.402S/\{1.02(1+788/[Na^+]^2)+S\}+0.0477S$, where v=influx in μmol/min, g final wet wt of slices; $[Na^+]$= concentration of Na^+ ions in medium, in mM; and S=concentration of α-amino*iso*butyric acid in medium, in mM. This equation shows two kinetically independent, parallel pathways of concentrative uptake: one, saturable and dependent on Na^+; the other, unsaturable and independent of Na^+. Influx is independent of ionic strength, Cl^- ion *per se*, and a moderate increase in tonicity. The binding of substrate to the saturable carrier depends on the Na^+ concentration; the maximum capacity of this carrier does not. For transport, $2\,Na^+$ ions must interact with each saturable transport site. This does not imply coupling between the flux of Na^+ and the flux of α-amino*iso*butyric acid.

The uptake of small organic molecules, and especially amino acids and sugars, by tissue preparations (brain slices, kidney slices, gut, muscle, etc.), by isolated cells (red blood cells, ascites cells, etc.), by microorganisms, and by subcellular preparations (mitochondria, synaptosomes, nuclei, etc.) is often partially or completely dependent on the presence of sodium ions. Much of the evidence and theory up to 1969–1970 is discussed in an excellent critical review by Schultz and Curran (1970). Various aspects are considered in reviews by Kimmich (1973), Kotyk (1973), Christensen et al. (1973), and Wilbrandt (1975). In my own area of interest, transport in the nervous system, an effect by sodium ions on the uptake of amino acids has been repeatedly demonstrated in vitro. Despite much research with many different preparations including brain slices, spinal cord slices, retina, vertebrate and invertebrate nerve, cultured tissues, cultured cell lines, and synaptosomes and other subcellular particles, there are only a few detailed studies of the effect of sodium ions on the kinetics of influx of amino acids. Important studies include: uptake of L-glutamate by crab nerve (Baker & Potashner, 1971; Evans, 1973); uptake of L-glutamate, L-aspartate and glycine by synaptosomes (Bennett, Logan & Snyder, 1973); uptake of L-glutamate by cockroach nerve cord (Evans, 1975); uptake of GABA [1] by synaptosomes (Martin, 1973); uptake of L-glutamate by peripheral nerve (Wheeler, 1976); and uptake of L-glutamate by synaptosomes (Wheeler & Hollingsworth, 1978). Of these, only Wheeler (1976), and Wheeler and Hollingsworth (1978) give explicit rate equations with numerical constants for the rate of uptake as a function of both sodium ion concentration and substrate concentration. No rate equations containing Na^+ dependence have been reported for brain slices, a widely used preparation of central nervous system tissue. The situation is little better with non-neural materials. Only a small fraction of the many studies of the dependence of amino acid transport on Na^+ ions has produced explicit rate equations.

In this study a rate equation that includes the explicit dependence on the concentration of Na^+ ions in the incubation medium was determined for the influx of AIB into slices of mouse cerebrum incubated at 37 °C. This synthetic amino acid was used to avoid complications from metabolism. Brain slices commonly have high capacity "low affinity" uptake sys-

[1] *Abbreviations:* GABA, γ-aminobutyric acid; AIB, α-amino*iso*-butyric acid; *N*-methyl-AIB, *N*-methyl-α-amino*iso*butyric acid; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; $tris^+$, tris(hydroxymethyl)methylammonium ion; cycloleucine, 1-aminocyclopentane-1-carboxylic acid; BCH, 2-aminobicyclo-(2,2,1)heptane-2-carboxylic acid.

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tem with K_t in the order of 1 mM for many amino acids. In addition they may have low capacity "high affinity" systems with much smaller K_t's for certain amino acids that are believed to be neurotransmitters. In agreement with our other studies of the kinetics of the low affinity systems for several amino acids under a variety of experimental conditions (S.R. Cohen, 1973, 1975, and *in preparation*), a range of substrate concentrations (0.2 to 20 mM) was chosen to permit the low affinity system to be investigated with only a negligible contribution from any high affinity systems that may exist for AIB. The brain slices were preincubated with medium to let water and electrolytes reach a more or less stationary state. Studies like the present one are not comparable to studies such as those by Kanner (1978) and Lajtha and Sershen (1975*a*) of influx in conjunction with an appreciable net flux of Na^+ ions.

Methods and Materials

Methods

The procedures used in our other studies of the kinetics of amino acid influx into brain slices (S.R. Cohen, 1973, 1975, and *in preparation*) were followed. Six- to nine-week-old Swiss mice were decapitated and the brain rapidly removed. Olfactory bulbs and underlying white matter were trimmed from the cerebral hemispheres, which were then immersed in chilled *standard* (*Na-133*) medium (below) for a few seconds. The hemispheres were removed from the medium, blotted, and slices, nominally 0.37 mm thick, were cut as described by Blasberg and Lajtha (1965) using a calibrated (Cohen, 1974*a*) McIlwain-Buddle (1953) tissue chopper. Most runs were made with males even though no difference between the sexes was observed in our first study (Cohen, 1973). Slices from one hemisphere (about 125 to 150 mg) were placed in 4.5 ml of oxygenated, substrate-free medium at 37 °C in a 25-ml stoppered erlenmeyer flask, and preincubated for 30 min in a thermostatted reciprocating water bath. One-half ml of medium at 37 °C containing [1-^{14}C]AIB with carrier at a concentration 10 times the desired concentration was quickly added and incubation was continued. After a predetermined interval the slices were rapidly filtered off with suction. The resulting tissue pellet was frozen in solid CO_2, weighed, homogenized in 5% (wt/wt) perchloric acid, and the concentration of AIB determined by liquid scintillation counting as described previously[2] (Cohen et al., 1968, 1970). With *Na-30*, *Na-14*, *Na-14/choline*, and *Na-14/HT* media (below) uptake was determined as a function of incubation time for each concentration of AIB (Fig. 1). The rate of influx was calculated from the slope of the linear portion of the graph. With *Na-60* medium the linear range and the rate were determined for 0.2, 2, and 20 mM AIB. At other concentrations of AIB, tissue was incubated for either 2 minutes or 5 minutes, both periods within the linear range; the rate was calculated from the increase in AIB in tissue over this interval. These procedures were adopted to eliminate the contribution from substrate entering the *functional* extracellular space (Cohen, 1972) for AIB uptake, because this tissue compartment cannot be measured at present. When the rate was calculated from the slope of a plot, each data point was determined in triplicate, and a straight line was fitted by least squares on y using at least four sets of data points; when the rate was calculated from the uptake at two incubation periods, six replicates were made of each data point.

Chemicals. [1-^{14}C]AIB was from New England Nuclear Corp. (Boston, Mass.). It gave a single spot with ascending paper chromatography using butanol-acetic acid-water (60:15:25 by volume) and methanol-pyridine-water (80:4:20 by volume) as solvents. Other chemicals were reagent grade or the best available from various sources.

Media. Standard medium (*Na-133*): 119 mM NaCl, 5.0 mM KCl, 0.75 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.0 mM NaH_2PO_4, 1.0 mM $NaHCO_3$, 25 mM HEPES, 12 mM NaOH (which reacts to form the complementary base of the buffer), and 10 mM glucose. This medium (Cohen et al., 1970) was used in our earlier studies (Cohen, 1973, 1975) of the kinetics of amino acid uptake. The concentration of Na^+ is 133 mM. *Na-60*: 46 mM NaCl, 146 mM sucrose, other components unchanged. *Na-30*: 16 mM NaCl, 206 mM sucrose, other components unchanged. *Na-14*: No NaCl, 238 mM sucrose, other components unchanged. *Na-14/choline*: No NaCl, 119 mM choline chloride, other components unchanged. *Na-14/HT* (high tonicity): No NaCl, 301 mM sucrose, other components unchanged. *Na-138/pyruvate*: 5 mM Na pyruvate, no glucose, other components unchanged. (Results in *standard* medium and *Na-138/pyruvate* medium that are cited in the text come from other studies.)

Curve Fitting and Precision. Eqs. (1) and (6) were fitted to the data to give the parameters in Tables 1 and 3 by an iterative procedure in which the sum of the squares of the relative errors[3] was minimized. (*See* Cohen (1968) for a justification of this criterion of best fit.) The relative standard deviation[4] of individual data points was taken as the measure of the fit and listed in the appropriate table. The precision of V_{max}, K_t, and k_u Eq. (1) was not estimated because they are not linearly independent, and because Barber, Welch, and MacKay's (1967) assumption about the nature of errors in V_{max} and K_t does not apply. Their precision should be about the same as in our earlier study of the influx of AIB (Cohen, 1973), i.e., V_{max}, ±10%; K_t, ±5%; and k_u, ±10%. During curve fitting the ratio $k_1 = V_{max}/K_t$ changed much less than either V_{max} or K_t did individually. Therefore it is much better determined by the data and probably has a precision of ±1 to 2%.

Rates were computed in units of μmol/g final wet wt of tissue, min instead of μmol/g intracellular water, min because the *functional* extracellular space is not known. This does not affect K_t or K_{Na}; it decreases V_{max} and k_u by the factor (g intracellular water)/(g final wet wt of tissue).

Results

Dependence of Influx on AIB Concentration

Brain slices incubated in any of the media, even those containing only 14 mM Na^+, concentrate AIB (Fig. 1). The rate of uptake fits the equation

$$v = V_{max} S/(K_t + S) + k_u S \quad (1)$$

[2] No corrections for counts in metabolic products are necessary because AIB is not metabolized by brain slices (S.R. Cohen, *in preparation*).

[3] RE=(observed value − calculated value)/(calculated value).

[4] $RSD = [\Sigma(RE)^2/(\text{number of data points} - \text{number of parameters})]^{\frac{1}{2}}$. The number of parameters is 3 for Eq. (1) and 2 for Eq. (6).

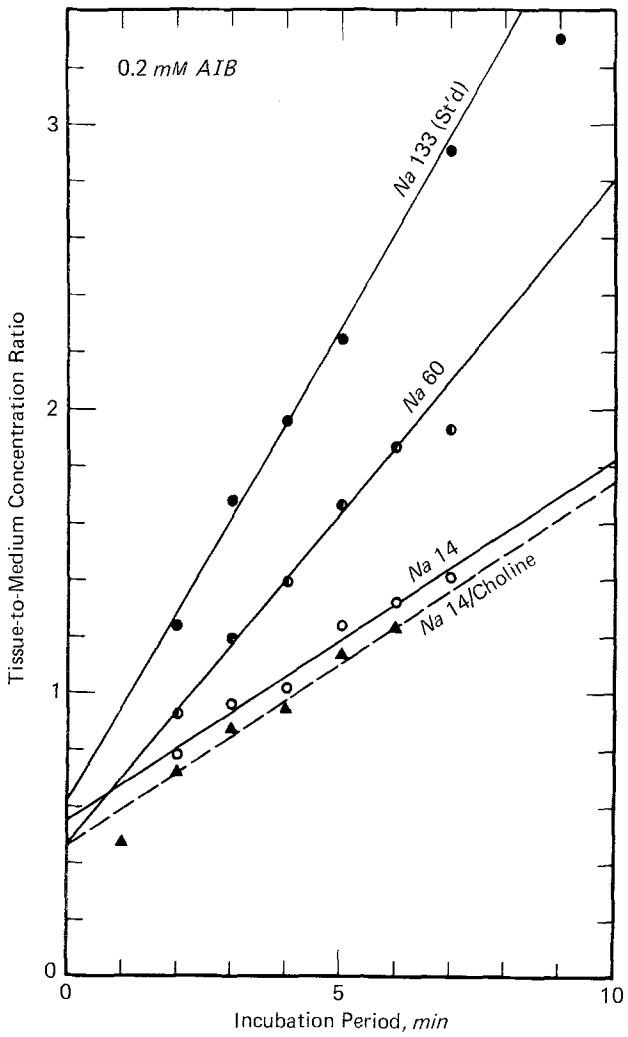

Fig. 1. Uptake of AIB by brain slices in media containing different concentrations of Na^+. Concentrations of AIB in medium is 0.2 mM. Units of tissue-to-medium concentration ratio are (μmol/g final wet wt of tissue)/(mM in medium). ●, *Na-60* medium; ◐, *Na-30* medium; ○, *Na-14* medium; ▲, *Na-14/choline* medium

where v is the rate, S is the concentration of AIB in the medium, and V_{max}, K_t, and k_u are constants (Fig. 2). Values for the constants are given in in Table 1. The right-hand side of this equation consists of terms for two kinetically distinct transport systems in parallel. The first, represented by $V_{max}S/(K_t+S)$, is saturable, can produce concentrative uptake, and follows Michaelis-Menten kinetics; the second is unsaturable, can also produce concentrative uptake, and follows simple first-order kinetics. The evidence that the second is properly represented by k_uS, and not by the kinetic expression for "passive diffusion", $k_D(S-S_i)$, where S_i is the intracellular concentration of substrate, is the same as in our other studies (S.R. Cohen, 1973, 1975, and *in preparation*), namely: (i) Even though influx by this system is important, ranging from 16 to 70% of the total in the concentration range studied (Table 2), graphs of uptake as a function of time (Fig. 1) have well defined linear portions where S_i increases greatly, often from less than S to appreciably greater; and (ii) Although S_i was greater than S for many of the measured rates, k_u is positive.

From 30 to 133 mM Na^+, the fractional contribution of the first-order unsaturable component to influx of AIB at concentrations up to 20 mM is almost independent of the Na^+ concentration (Table 2). The contribution of this component is markedly greater, however, at the lowest Na^+ concentration employed, 14 mM. This component is also an important pathway for AIB influx from *standard* medium at temperatures from 20 to 45 °C (Cohen, 1973) and from *Na-138/pyruvate* medium (S.R. Cohen, *in preparation*), and for the influx of a number of amino acids into preparations of various tissues and isolated cells (Cohen, 1973, 1975).

The Effect of the Replacement for Sodium Chloride

The influx of AIB is the same from *Na-14* medium, where NaCl is replaced by sucrose, 2 moles/mole, from *Na-14/choline* medium, where NaCl is replaced by choline chloride, 1 mole/mole, and from hypertonic *Na-14/HT* medium, where NaCl is replaced by sucrose, 2.5 moles/mole (Fig. 2). This agrees with the observation by Nakazawa and Quastel (1968) that partial or complete replacement of NaCl by sucrose, 2 moles/mole, or by choline chloride, 1 mole/mole, has the same effect on the 60-min uptake of glycine by rat brain slices. Several important conclusions follow: (i) These studies give the true dependence of the rate of the concentration of Na^+ ions in the medium. (ii) The rate is independent of the concentration of Cl^- ions. (iii) The rate is independent of the ionic strength[5] of the medium, at least over the range 27 to 146 mmol/liter. (iv) The rate is also unaffected by a 20% increase in tonicity.

Studies of the effect of Na^+ ions on biological processes are complicated because "non-sodium", a monovalent cation with no biological effects, does not exist. The possible substitute cations: $choline^+$, $tris^+$, Li^+, NH_4^+, K^+, Rb^+, Cs^+, Tl^+, $N(CH_3)_4^+$, etc., are all known to affect living systems. The uptake of various amino acids by brain slices incubated in low sodium or sodium-free media depends on whether the NaCl has been replaced by tris · Cl or KCl (Joanny et al., 1973), or by LiCl, RbCl, choline · Cl,

[5] Ionic strength $I=\frac{1}{2}\Sigma C_i z_i^2$, where C_i is the concentration of the ith species of ion and z_i is the charge on that ith species.

Table 1. Effect of concentration of Na^+ ions in medium on the kinetic parameters for the uptake of α-amino*iso*butyric acid by mouse cerebrum slices[a]

	Medium				Average
	Standard[b]	Na-60	Na-30	Na-14	
Concentration of Na^+ ions (mM)	133	60	30	14	
V_{max} (μmol/min, g final wet wt)	0.404	0.514	0.302	0.387	0.402 ±0.044
K_t (mM)	1.11	1.51	1.46	4.94	1.80 ±0.90
$k_1 = V_{max}/K_t$	0.362	0.340	0.297	0.0782	
k_u (μmol/min, g final wet wt, mM AIB in medium)	0.0528	0.0566	0.0357	0.0458	0.0477 ±0.004
Relative SD of a data point (percent)		6.8	5.9	6.6	

[a] Rate equation: $v = V_{max}\, S/(K_t + S) + k_u\, S$ [Eq. (1)].
[b] Values in *standard* medium are from Cohen (1973).

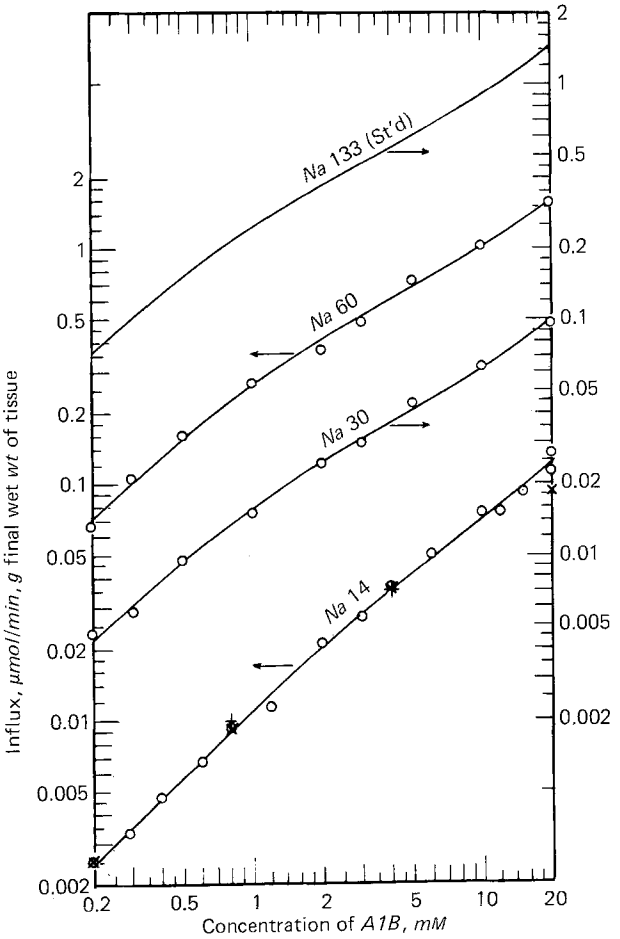

Fig. 2. Influx of AIB into brain slices as a function of its concentration in the medium. Curves are computed from Eq. (1) using kinetic parameters in Table 1. *Note:* For *Standard* medium, read influx on RH scale; for *Na-60* medium, read influx on LH scale; for *Na-30* medium, read influx on RH scale, multiply rate by 10; for *Na-14*, read influx on LH scale, multiply rate by 10. ×, *Na-14/choline* medium; +, *Na-14/HT* medium

Table 2. Percentage of total influx carried by the unsaturable system

Medium	Concentration of Na^+ in medium (mM)	Concentration of AIB in medium (mM)			
		0	0.2	2	20
Standard	133	13	15	29	73
Na-60	60	14	16	28	70
Na-30	30	15	16	29	72
Na-14	14	37	38	45	75

The values are calculated from kinetic parameters in Table 1.

or mannose (Lajtha & Sershen, 1975*b*). If the specific effects of other cations are avoided by simply reducing the NaCl content of the medium, its Cl^- content, its ionic strength, and its tonicity are correspondingly reduced; if NaCl is replaced by sucrose or some other "inert" nonelectrolyte, 2 moles/mole, the ionic strength is reduced although the tonicity is maintained. In addition, the nonelectrolyte may affect the preparation. Consequently, unless at least two substituents are used, studies of the effect of Na^+ ion may only be studies of the effect of Na^+ ion compared with $choline^+$ ion, or the effect of NaCl compared with sucrose.

Even though the rate is the same in these two 14 mM Na^+ media, choline chloride and sucrose are not equivalent. The linear region in graphs of uptake as a function of time (Fig. 1) is shorter and less clearly defined when NaCl is replaced by choline chloride. This may indicate that cell membranes are not maintained as well. At low concentrations choline is concentrated by brain slices (Schuberth et al., 1966), and at high concentrations it enters all tissue water freely from a hypertonic medium (Cohen & Lajtha, 1970); whereas at low concentrations (Cohen, 1974*b*; Cohen et al., 1970; Navon & Lajtha, 1969) and pre-

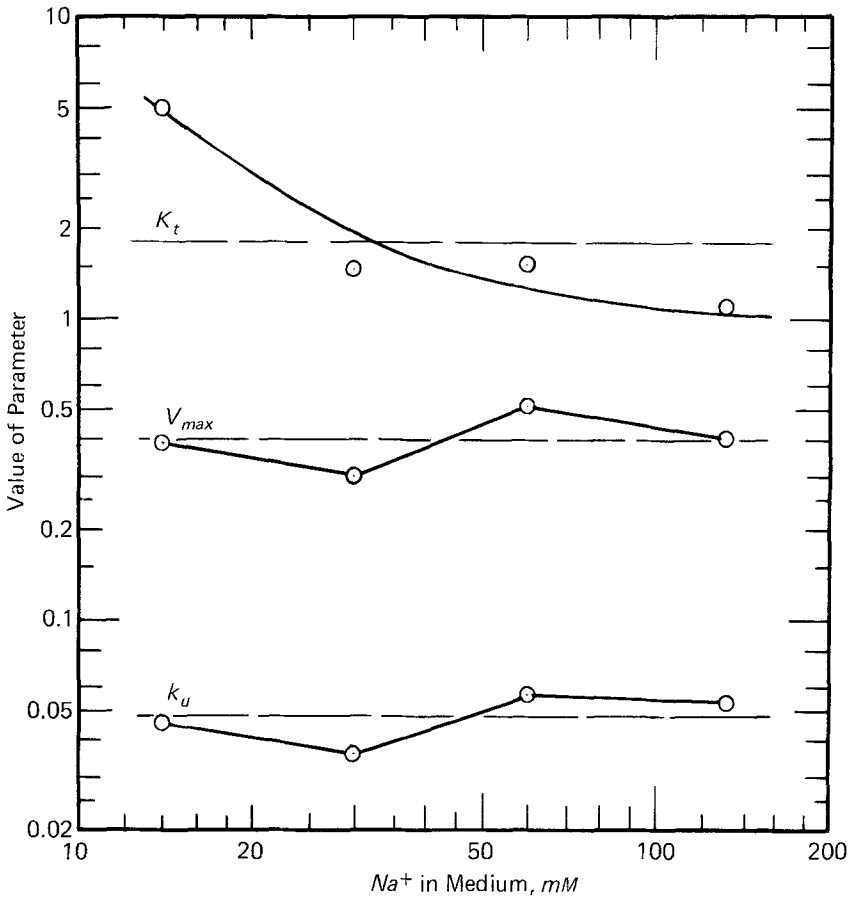

Fig. 3. Values of kinetic parameters as a function of concentration of Na^+ in medium. Units of K_t are mM; units of V_{max} are μmol/min, g final wet wt of slices; units of k_u are μmol/min, g final wet wt of slices, mM substrate in medium—— average value.

sumably also at high concentrations, sucrose is excluded from a sizable fraction of the tissue water.

Dependence of Influx on Na^+ Concentration: The Complete Rate Equation

The unsaturable transport system is not affected by the concentration of Na^+ in the medium in the range 14 to 133 mM. The total variation of k_u is small and shows no obvious relation to the Na^+ concentration (Table 1; Fig. 3).

The rate of uptake by the saturable transport system decreases as the Na^+ concentration is reduced over the above range. The parameter V_{max}, which is a measure of the maximum influx by this component, is more or less constant; the variations show no obvious relation to the Na^+ concentration (Table 1; Fig. 3). The Michaelis constant K_t, which is approximately equal to $1/K_{binding}$ where $K_{binding}$ is the equilibrium constant for association of substrate with the carrier[6], increases markedly at low Na^+ concentrations (Table 1; Fig. 3). Therefore decreasing the Na^+ concentration in the medium does not alter the *capacity* of this transport system, but reduces influx by weakening the binding of the substrate to the carrier.

The following simple model was used to find the complete rate equation. Assume that the carrier for the saturable component exists in two forms: C, the form in high-Na^+ media; and C', the form in low-Na^+ media, which are in equilibrium according to the relation

$$C \rightleftharpoons C' + n\mathrm{Na}^+ \quad (C = \mathrm{Na}_n\, C'). \tag{2}$$

If n is 2 or more, this model assumes that the fraction of carrier in the intermediate forms NaC, $\mathrm{Na}_2 C$, ..., $\mathrm{Na}_{(n-1)} C$ is insignificant (*see* Wheeler & Hollingsworth, 1978). Let $k_1 \equiv V_{max}/K_t$ be the first-order rate constant at infinitely low substrate concentration for the equilibrium mixture in a given medium; let $k_1{}^0$ be the first-order rate constant for C; and let k_1' be the first-order rate constant for C'. (The first-order rate constant was used because it is much better defined by the data than either V_{max} or K_t are.) Let F be the fraction of C in the equilibrium mixture.

[6] The term "carrier" is used for convenience. No specific mechanism is implied.

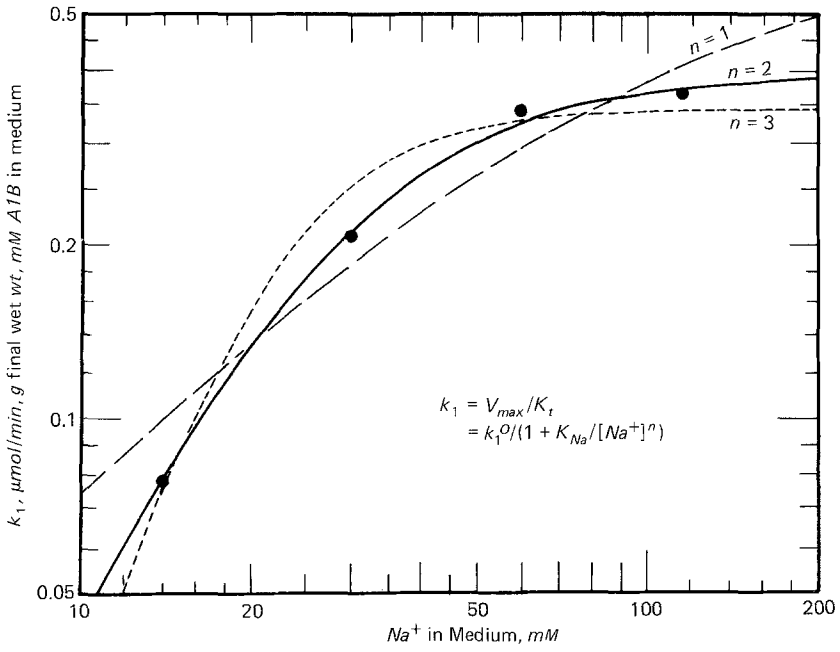

Fig. 4. The apparent first-order rate constant $k_1 = V_{max}/K_t$. Curves are computed from Eq. (6) using constants in Table 3 assuming $n = 1$, 2, or 3

Table 3. Fit of data to equation $k_1 = k_1^0/(1 + K_{Na}/[Na^+]^n)$

	$n=1$	$n=2$	$n=3$
k_1^0	0.708	0.394	0.343
K_{Na}	85	788	9800
Relative SD (%)	24	4.8	14

Equation fitted to values of $k_1 = V_{max}/K_t$ from Table 1. Units of $[Na^+]$ are mM; units of k_1 and k_1^0 are μmol/min, g final wet wt, mM AIB in medium.

Table 4. Fit of Eq. (9) to data

	Medium			
	Standard	Na-60	Na-30	Na-14
Concentration of Na^+ ions (mM)	133	60	30	14
$k_1 = V_{max}/K_t$ observed (from Table 1)	0.362	0.340	0.207	0.0782
$k_1 = V_{max}/K_t$ calculated [from Eq. (9)]	0.377	0.323	0.210	0.0783
Relative deviation (%) = $100\,(k_{1_{abs}} - k_{1_{calc}})/k_1$	−4.0	+5.3	−1.4	−0.1

Then

$$k_1 = k_1^0\, F + k_1'(1-F) \tag{3}$$

and

$$[Na^+]^n[C']/[C] = [Na^+]^n(1-F)/F = K_{Na} \tag{4}$$

where $[Na^+]$ is the concentration of Na^+, and K_{Na} is the equilibrium constant for the dissociation reaction Eq. (2). Combining Eqs. (3) and (4) gives

$$k_1 = (k_1^0 - k_1')/(1 + K_{Na}/[Na^+]^n) + k_1'. \tag{5}$$

This is an equation with 4 adjustable constants, with the restrictions from the model that they cannot be negative, that k_1' must be less than k_1^0, and that n must be an integer. Preliminary fits showed that uptake by the low-Na^+ form is negligible; k_1', for example, is less than 3% of k_1^0 with n assumed to be 2. Therefore k_1' was assumed to be 0, giving Eq. (6).

$$k_1 = k_1^0/(1 + K_{Na}/[Na^+]^n) \tag{6}$$

which was fitted to the data with the results shown in Table 3 and Fig. 4. Excellent agreement was obtained with $n=2$. The dependence of the saturable component on the concentration of Na^+ ions in the medium is consistent with a cooperative 2-Na^+ ion process that converts the inactive form of the carrier to the active form. From the values in Table 3 for $n=2$, $k_1 = 1\%$ of k_1^0 with 2.8 mM Na^+; 5% with 6.4 mM Na^+; 10% with 9.4 mM Na^+; 20% with 14 mM Na^+; 50% with 28 mM Na^+; 80% with 56 mM Na^+; and 96% with 133 mM Na^+, the concentration in standard medium. Since V_{max} does not depend on

the concentration of Na^+, it follows from Eq. (6) with $n=2$ and the definition of $k_1 = V_{max}/K_t$, that

$$K_t = K_t^0(1 + K_{Na}/[Na^+]^2) \quad (7)$$

where $K_t^0 = V_{max}/k_1^0$ is the limiting value of K_t at infinitely high Na^+ concentration. Calculated values of K_t using the average value of V_{max} in Table 3 are shown in Fig. 4. Combining Eqs. (1) and (7) gives the complete rate equation

$$v = V_{max}\, S/\{K_t^0(1 + K_{Na}/[Na^+]^2) + S\} + k_u\, S. \quad (8)$$

With numerical values for the constants this is

$$v = 0.402\, S/\{1.02(1 + 788/[Na^+]^2) + S\} + 0.0477\, S \quad (9)$$

with concentration expressed in mM and influx in μmol/min, g final wet wt of slices. It is apparent from Table 4 that Eq. (9) fits the data closely.

Discussion

The aim of this study was to determine the phenomenological rate equation for the relation between influx of a particular amino acid into a selected tissue preparation and the concentrations of substrate and of sodium ions in the incubation medium. No assumptions about mechanisms were made beyond those implied by a simple and only slightly restrictive model. Such rate equations are valuable in themselves—all too few are known—and provide a necessary guide for "model builders", but more should not be read into them than they contain. By themselves, they permit a wide variety of interpretations.

The "Ionic Atmosphere" and Specific Effects

The data show that influx is insensitive to large changes in the "ionic atmosphere"—apart from effects of specific ions—and to moderate changes in tonicity. This is surprising because the general ionic atmosphere, of which the ionic strength is a measure, affects a wide variety of fundamental physical parameters, such as thermodynamic activities, electrostatic interactions between ions, ζ-potentials at cell membranes, and so on. It may be that some observed differences between ionic and non-ionic substitutes for NaCl show specific effects on transport systems that are basically insensitive to the ionic atmosphere.

When NaCl is replaced by a nonelectrolyte it is generally assumed that any observed effects are due to changes in Na^+ ion concentration even though Cl^- ions can cross plasma membranes. Peterson and Raghupathy (1978) found little effect on the accumulation of serine and threonine by synaptosomes when NaCl was replaced by Na_2SO_4 (0.5 mole/mole) with sucrose added to maintain tonicity. Transport may still depend on the anion accompanying Na^+ even when this counter ion has no direct effect in itself. When chloride ion is replaced by mucate ion, $^-OOC(CHOH)_4COO^-$, or by toluene-2,4-disulfonate ion, influx of glycine into pigeon red blood cells is greatly reduced because these ions, which cannot enter the cells, hold back Na^+ ions that accompany glycine (Vidaver, 1964*b*).

Saturable and Unsaturable Uptake

A two-term rate equation [Eq. (1)] for saturable and unsaturable transport is parallel has been found for the uptake of several amino acids by brain slices incubated under a variety of conditions: AIB at temperatures from 20 to 45 °C (Cohen, 1973), L-valine and GABA at 25 and 37 °C, L-lysine at 30 and 37 °C (Cohen, 1975) and L-leucine at 37 °C (S.R. Cohen, *unpublished*), all from *standard* medium; AIB at 37 °C, and GABA at 25 and 37 °C from a medium (*Na-138/pyruvate*) containing sodium pyruvate instead of glucose as the energy source (S.R. Cohen, *in preparation*). Two kinetically distinct systems for influx operating in parallel, one saturable with Michaelis-Menten kinetics, and one "passive diffusion" or unsaturable with first-order kinetics, have been reported for a large number of other *in vitro* systems. [*See* Cohen (1975) for examples.] Despite having the same rate equation, studies of the effect of changing the incubation temperature or the composition of the medium (Cohen, 1975, and *in preparation*), and measurements of the coupling between the flux of substrate and the flux of Na^+ ions (Stallcup, Bulloch & Baetge, 1979) have shown qualitative differences in both the saturable systems and the unsaturable systems. In much of the literature it is impossible to tell whether the unsaturable component that is reported is first order with a rate given by $k_u S$ or passive diffusion with a rate given by $k_D(S - S_i)$. Many of the experiments were not designed to distinguish between them. The workers are frequently confused on this point; they may refer to the process as "passive diffusion" but treat it as a first-order process. Since these processes often carry an appreciable fraction of the total influx and since in some cases they are known to carry substrate against a concentration gradient, they should not be dismissed as merely "passive diffusion" as is sometimes done. In the absence of evidence to the contrary, it might be best to consider them to be active transport systems.

Table 5. Effect of reduced Na^+ ion content of medium on V_{max} and K_t

Effect on V_{max}	None	Decrease	Decrease	Decrease	None
Effect on K_t	Increase	None	Increase	Decrease	None
Amino Acid					
AIB	This study		G, H, I		
N-methyl-AIB			G		
Glycine	N, O, R	M	B, C	D	
L-alanine	R		I		
L-serine			B		
L-methionine	N		G		G
L-valine				B	R
L-leucine					R
L-isoleucine					F
L-phenylalanine			I, J	F	
L-tyrosine			J		
L-glutamic acid	A, E, P, Q			B	
β-alanine		B			
GABA			K		
Cycloleucine		H		L	
BCH		L			

A Crab peripheral nerve. Baker & Potashner (1971).
B Pigeon red blood cells. Eavenson & Christensen (1967).
C Mouse ascites-tumor cells. Eddy et al. (1967).
D Bone. Finerman & Rosenberg (1966).
E *Escherichia coli*. Halpern et al. (1973).
F Cultured human skin fibroblasts. Hillman & Otto (1974).
G Ehrlich ascites cells. Inui & Christensen (1966).
H Ehrlich ascites cells. Jacquez (1973).
I Ehrlich ascites cells. Jacquez et al. (1970).
J Rat cerebral cortex slices. Joanny et al. (1973).
K Synaptosomes. Martin (1973).
L Ehrlich ascites cells. McClellan & Schafer (1973)
M Synaptosomes. Peterson & Raghupathy (1973).
N Ehrlich ascites cells. Potashner & Johnstone (1971).
O Pigeon red blood cells. Vidaver (1964*a*).
P Frog peripheral nerve. Wheeler (1976).
Q Synaptosomes. Wheeler & Hollingsworth (1978).
R Rabbit red blood cells. Wheeler & Christensen (1967*a*).

The Rate Equation and Coupling Between Amino Acid and Na^+ Ion Fluxes

The dependence of the saturable influx on $[Na^+]^2$ does not imply that $2\,Na^+$ ions enter per molecule of AIB taken up by this path in addition to any Na^+ ions that enter in the absence of AIB. The $2\,Na^+$ ions may act at an immobile site. If they act on a mobile carrier, they may not be discharged or may be incompletely discharged with the AIB molecule; alternately, they may be discharged even when the AIB molecule is recaptured and returned to the outside of the cell. Although Vidaver (1964*c*) found agreement in pigeon red blood cells between the rate equation for the saturable influx of glycine, which indicates a dependence on $[Na^+]^2$, and a flux ratio of about 2:1; and Schafer and Jacquez (1967) found a flux ratio of 1:1 over a wide variety of conditions for the saturable uptake of AIB by ascites cells, where, presumably, influx is a function of the first-power of $[Na^+]$; there is in general no correlation between the rate equation and the flux ratio (Wheeler et al., 1965; Schultz & Curran, 1970; Geck, Heinz & Pfeiffer, 1972). Wheeler and Christensen (1967*b*) found a flux ratio of about 2.5:1 with a first-power dependence on $[Na^+]$ for the uptake of alanine by pigeon red blood cells. Depending on the amino acid and the preparation, flux ratios from 0.2 to 5 have been reported for the saturable uptake of amino acids by systems for which kinetic data indicate the interaction of $1\,Na^+$ ion with each carrier (Christensen et al., 1973).

The Effect of Na^+ Ions on k_u, V_{max}, and K_t

A wide variety of patterns have been reported for the effect of reduced Na^+ ion concentration on k_u, V_{max}, and K_t. There is no obvious correlation with preparation, amino acid, or experimental condition. In addition to the system reported here, an unsaturable Na^+-independent component of influx (or Na^+-independent "passive diffusion"[7]) has been reported for L-glutamate in crab (*Carcinus maenas*) peripheral nerve (Evans, 1973), spider crab (*Maia squinada*) peripheral nerve (Baker & Potashner, 1971), and cockroach (*Periplaneta americana*) abdominal nerve cord (Evans, 1975); for glycine in pigeon red blood cells (Vidaver, 1964*a*); for AIB and *N*-methyl-AIB in Ehrlich ascites cells (Inui & Christensen, 1967); and for L-isoleucine in cultured human-skin fibroblasts (Hillman & Otto, 1974). An unsaturable component that is Na^+ dependent has been reported for the influx of L-phenylalanine into Ehrlich ascites cells (Jaquez, Sherman & Terris, 1970).

Table 5 contains examples of the different effects that reduced Na^+ ion content of the medium has been reported to have on the saturable component. As in the present study, V_{max} may be unchanged and K_t be increased. Alternately V_{max} may be decreased and K_t may remain unchanged. V_{max} may be decreased and K_t be increased, both changes serving to diminish influx. Both V_{max} and K_t may be decreased, with the change in V_{max} being more than sufficient to overcome the change in K_t. Finally both constants may be unchanged. For several amino acids more than one pattern has been reported, depending on the prepara-

[7] For the reasons given above and in Cohen (1975), I am considering "passive diffusion" to actually be unsaturable concentrative uptake.

tion or the worker. In the absence of Na^+ ions in the medium there is little or no uptake of many amino acids by a saturable process. This agrees with the present study and corresponds to $k_1'=0$ in Eq. (3). There are many exceptions. Besides the instances in Table 5, saturable uptake in the absence of Na^+ ions has been reported for several diverse systems, including glycine entering synaptosomes (Peterson & Raghupathy, 1973), AIB entering fetal rat calvaria (Finerman & Rosenberg, 1966), and L-histidine into *S*37 ascites tumor cells (Matthews, Leslie & Sholefield, 1970). It is often difficult or impossible to ascertain from the literature whether the observed influx was produced by a sodium-insensitive system, a sodium-dependent system which retains some activity in sodium-free media, or two systems, one sodium-dependent, the other sodium-insensitive.

The functional dependence of saturable influx on the concentration of Na^+ ions in the medium is also diverse. Most studies, where there are sufficient data, indicate that influx is more or less a function of the first power of $[Na^+]$ (Eddy, Mulcahy & Thomson, 1967; Inui & Christensen, 1966; Wheeler & Christensen, 1967*b*; Thomas & Christensen, 1971; Evans, 1973, 1975; Sprott et al., 1975; Wheeler, 1976). Some, like the present one, indicate that it is largely or entirely a function of $[Na^+]^2$ (Vidaver, 1964*a*; Baker & Potashner, 1971; Peterson & Raghupathy, 1972, 1973; Wheeler & Hollingsworth, 1978). Wheeler and Christensen (1967*a*) found both first-power and second-power terms necessary, with the second-power terms more important, to express the effect of Na^+ concentration on the influx of glycine into rabbit reticulocytes; the rate followed the relation $v=C_1[Na^+]^2/(C_2+C_3[Na^+]+[Na^+]^2)$, where C_1, C_2 and C_3 are functions of the glycine concentration.

It is of some interest to compare the present study with several other detailed studies of the kinetics of the Na^+-dependent uptake of amino acid by neural tissues and other preparations, even though, in most cases, an explicit rate equation analogous to Eq. (8) or (9) is not presented. In all of the following studies the Na^+-dependent uptake showed Michaelis-Menten kinetics. There are four important studies of the Na^+-dependent component of L-glutamate influx into nerve preparations. Dependence on the first power of $[Na^+]$ was found by Wheeler (1976) for bullfrog (*Rana catesbeiana*) sciatic nerve, and by Evans for peripheral nerves from the walking legs from crabs (*C. maenas*) (1973) and for cockroach (*P. americana*) abdominal nerve cord (1975). Wheeler found that K_t was dependent on $[Na^+]$ while V_{max} was unaffected and furthermore showed that his observations were consistent with the reaction of inactive carrier with Na^+ to form a 1:1 complex which transported glutamate. Evans (1973, 1975) does not state whether V_{max}, K_t, or both are sodium dependent. By contrast, Baker and Potashner (1971) found a dependence on $[Na^+]^2$ for uptake by peripheral nerves from the walking legs of spider crabs (*M. squinado*). They also observed inhibition by K^+ ions that was dependent on the second power of $[K^+]$. They derived the rate equation

$$v=V_{max}[Glu]/\{K_{Glu}[(K_1K_2/[Na^+]^2)([K^+]^2/K_3K_4 + [K^+]/K_3+1)+K_2/[Na^+]+1+[Glu]\}.$$

Although they claim to have used it to fit smooth curves to their data, they do not give numerical values for the constants. For the uptake of glycine by mouse ascites-tumor cells Eddy et al. (1967) developed the theoretical rate equation

$$v=[Na^+]\,S(k_e/k_1k_4)/\{1+[Na^+]/k_1+[K^+]/k_2 + S(1/k_3+[Na^+]/k_1k_4+[K^+]/k_3k_7)\}$$

[Eq. (8) in this reference], and evaluated the constants. They proposed that either one Na^+ ion or one K^+ ion could interact with the carrier, and that although a ternary complex (glycine, carrier, K^+) could form, glycine could only be transported as the ternary complex (glycine, carrier, Na^+).

Wheeler and Hollingsworth (1978) determined the kinetics for the "high affinity" uptake of L-glutamate by synaptosomes prepared from the cerebral cortex of adult Long-Evans rats and found K_t to depend on $[Na^+]^2$. They derived rate equations for five assumed mechanisms including one, their Model IV, that gave an equation $v=V_{max}S/\{(K_2+K_1K_2/[Na^+]^2)+S\}$, which is the same as the saturable term in Eq. (8) of this study, and evaluated the constants for all five. Although they chose one, their Model III, both on theoretical grounds and because it gave the "best fit"[8] to data, all models gave excellent fits and there is little reason to choose any one on this basis. Bennett et al. (1973) present evidence that the "high affinity" uptake of L-glutamate by synaptosomes prepared from the cerebral cortex of adult Sprague-Dawley rats is a function of $[Na^+]$ to the first power although they do not indicate whether K_t, V_{max}, or both vary with sodium-ion concentration. Wheeler and Hollingsworth consider that this may represent a difference between the two strains of rats. It is likely that it represents differences between the mixture of subcellular particles termed "synaptosomes" prepared in different laboratories. Vidaver (1964*a*) found the influx of glycine into pigeon red blood cells to depend on the square of the Na^+-ion

[8] Constants were evaluated by minimizing $\Sigma(1/v_{observed}-1/v_{calculated})^2$ summed over all data points. *See* Cohen (1968) for comments on "best values" of Michaelis-Menten constants.

concentration according to the rate equation given by the term for saturable transport in Eq. (8). [*Compare* the present study with this study by Vidaver and with the studies by Baker and Potashner (1971), Eddy et al. (1967), Wheeler (1976), and Wheeler and Hollingsworth (1978).] Eavenson and Christensen (1967) confirmed this dependence for Na^+ concentrations above 20 mM, but found a significant contribution from first-power terms at lower Na^+ concentrations. The influx of L-alanine (Wheeler et al., 1965) and L-serine (Eavenson & Christensen, 1967) into the same preparation is a function of the first power of the Na^+-ion concentration.

Our studies of transport kinetics and the studies of others have revealed an almost infinite variety that, for the present at least, research cannot diminish nor custom stale. It is probably premature to consider a mechanism for amino acid uptake by any one preparation, let alone by several diverse preparations. It may be better policy to regard each combination of preparation and substrate as a species unto itself, and to patiently work out the kinetics.

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