

Mathematical Modelling of the Transport of Low Molecular Weight Solutes Across Biological Membranes. The Transport of Leu, His and Glu into Human Blood Platelets

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Abstract. A model describing the transport of low molecular weight solutes across cell membranes is presented. The model accounts for many different systems which may mediate the fluxes of various solutes, for the effect of Na^+ ions, and for time dependence of the processes. It generalizes the classical three-parameter equation for transport. Solutions to the model were employed to interpret experimental data obtained for the uptake of DL-leu, L-his and L-glu by human blood platelets.

Key words: Mathematical Modelling — Transport — Amino Acids

Introduction

The transport of solutes across biological membranes has been studied extensively in a number of cells derived from different species. Regardless of the cell source, the fluxes of solutes were observed to have some common features. The transfer of solutes is 1) associated with ionic pumps (Anraku 1982; Berteloot et al. 1982; Fujimura et al. 1983a,b; Hayashi et al. 1980; Hoshi and Himukai 1982; Klip et al. 1983; Ozbilen et al. 1980; Weigensberg et al. 1982), 2) to a large extent inhibited by metabolic inhibitors (Anraku 1982; Korpi 1983; Ozbilen et al. 1980; Suzuki 1981), and 3) saturable upon increasing concentrations of solutes (Anraku 1982; Franchi-Gazzola et al. 1982; Hoshi and Himukai 1982; Inui et al. 1983; Kontro and Oja 1981; Reynolds et al. 1982). On the other hand, transport systems could be found independent of ionic pumps and unsaturable in the concentration ranges of solutes used (Floud and Fahn 1981; Lussier et al. 1982; Rosenberg et al. 1980). Transport systems present in membranes may be specific for some solute. Some solutes, however, are transported by more than one system showing different mechanisms and binding affinities. Also, transport systems exist which can mediate fluxes of several solutes (Logan et al. 1982; Oxender et al. 1977; Shotwell et al. 1981; Yardimci et al. 1981a,b). Based on the similarities, two principally different types of transport of solutes across biological membranes have been postulated, namely diffusion and carrier transport.

Two commonly accepted general models have been developed describing phenomenological properties of the transport, the channel and the carrier transport (Deves and Krupka 1979a, b; Eilam and Stein 1974; Kotyk 1975; Lieb 1982). However, both these models have some flaws making them difficult to apply to interpretation of experimental data. For example, one of their assumptions is that the concentration of any carrier form is in steady-state. This imposes certain limitations on the practical use of the models. Usually, steady-state is reached after long incubation times and it is almost impossible to perform experiments under steady-state conditions. Another flaw of these models is that they consider only one transport system for each solute, though it is evident from experimental observations, in particular concerning amino acids, that there is a multiplicity of systems mediating fluxes of the solutes across the membrane (Logan et al. 1982; Oxender et al. 1977; Shotwell et al. 1981; Yardimci et al. 1981 a, b). Therefore, oversimplifications of the models seem too restrictive and the application of the models to describe exchange equilibrium may be misleading. Indeed, exchange equilibrium for a membrane transport, with several systems being operative simultaneously, should not be taken as the equilibrium typical of a particular system. It may happen that the solute is simultaneously transported by two systems in opposite directions. Then, both systems will be far from being in equilibrium although the total exchange is in equilibrium.

In this work we discuss the general situation when different solutes are transported by several systems. We shall propose a mathematical model describing the transport of amino acids and other low molecular weight solutes with a multiplicity of different systems being operative simultaneously in the transport of different solutes. This model facilitates the interpretation of experimental data which, due to technical limitations, are usually obtained for states far from equilibrium.

The usefulness of the model was tested on biological material. In order to precisely describe the kinetics of fluxes of amino acids across the membrane, both the kinetics of protein synthesis and the uptake of amino acids by cells should be considered. To some extent these difficulties may be overcome by using for transport studies relatively small cells with unsophisticated structure which do not synthesize proteins. Human blood platelets meet these requirements and they were thus used for our studies. Blood platelets do not contain nuclei, and the residual protein synthesis due to messenger RNA received with the cytoplasm from megakaryocytes is extremely limited (Booyse and Rafelson 1968; Plow 1979; Warshaw et al. 1967). In addition, owing to their small size blood platelets represent a convenient model to study the mechanism of amino acid transport.

Materials and Methods

Platelet isolation. Platelets were isolated according to the procedure described previously (Wal-kowiak and Cierniewski 1987).

Transport assay. DL-leucine (10.5 Ci/mol), L-glutamic acid (3.87 Ci/mol, from Institute of Nuclear Research, Swierk, Poland), and L-histidine (240 Ci/mol, from Institute for Research, Production and Application of Radioisotopes, Prague, Czechoslovakia) were used in the transport studies.

Usually, 100 μl of platelet suspension (2×10^9 platelets per ml) in buffer A (140 mmol/l NaCl, 5 mmol/l KCl, 10 mmol/l glucose, 0.1 g/l apyrase, and 15 mmol/l HEPES, pH 7.4) were mixed with 100 μl of the same buffer and then 10 μl of ^{14}C -amino acid were added. The final concentration of radiolabelled amino acids was 0.43 mmol/l in the incubation mixture. All amino acid uptake assays were carried out at 37°C. After incubation the mixtures were filtered immediately through a Synpor filter (0.45 μm , 25 mm), and washed once with 5 ml of ice-cold transport medium. In order to examine the extent of amino acid adsorption on the filter, 10 μl of radiolabelled amino acid were mixed with 200 μl of the transport medium and applied onto a filter. After washing the filter with the buffer it was dried and the radioactivity retained was measured in a counter (Polon ZM-701). The results are expressed in picomoles of amino acid taken up per 2×10^8 platelets.

The Model

Experimental data suggest the existence of multiple types of transport systems of solutes across biological membranes. The first group includes transport systems which depend on the concentration gradient of Na^+ ions. They show relatively high affinity for solutes and are efficient over low solute concentration ranges. These systems become saturated upon increasing solute concentrations. The second group are transport systems which although not requiring any Na^+ ion gradient, also have high affinities to solutes and are saturable. These systems are characteristic by asymmetry of binding affinities on both sides of the membrane for the formation and dissociation of complexes of the carrier protein with the solute. The third group includes systems independent of Na^+ ions and having relatively low affinities to solutes. They become saturated only at very high solute concentrations and are cell energy independent.

As far as the carrier protein is concerned, it is assumed to exist in two states, free and associated with the solute transported. Thus, the role of the carrier is merely that of membrane channel. In the first group of systems, this channel is under control of Na^+ ions, in the second group it is regulated by some unknown mechanisms, and in the third group, the regulation is entirely diffusion dependent.

In addition, we assume that the transport of solutes takes place within the membrane and its very close environment. In more distant areas, transport of solutes is controlled by diffusion laws and will not be considered. The ex-

tracellular concentrations of solutes change during the transport processes within the range of 10^{-4} — $10^{-1}\%$ (Cierniewski and Walkowiak 1983; Haschemeyer and Detrich 1982; Lussier et al. 1982; Rosenberg et al. 1980). This means that the extracellular concentration of solutes is effectively constant.

Different low molecular weight solutes may be simultaneously transported by several systems classified in these three groups. When a particular system does not mediate the transport of a particular solute, its affinity to the solute is zero.

We shall consider the transport of m different solutes across a membrane to be mediated by n different systems according to the mechanism shown in scheme (i)



where S_i^1 and S_i^2 is the i -th solute ($i = 1, \dots, m$) outside and inside the cell, respectively, B_j is the carrier protein involved in the functioning of the j -th transport system ($j = 1, \dots, n$), T_{ij} is the complex of the i -th solute with the carrier protein of the j -th transport system, k_{ij}^1 and k_{ij}^{-2} are rate constants of complex formation of the i -th solute with the carrier protein of the j -th transport system on the external and internal surface of the cell membrane, k_{ij}^{-1} and k_{ij}^2 are rate constants for dissociation of the complex T_{ij} on both sides of the cell membrane.

In addition to the above general properties, in all groups of the transport systems the following abbreviations will be used: if there are n transport systems within three groups, the systems marked by index $j = 1, \dots, r - 1$ correspond to the first group of systems, those by $j = r, \dots, p - 1$ to the second group, and those by $j = p, \dots, n$ to the third group of the transport systems.

Systems marked by $j = 1, \dots, r - 1$ are assumed to transfer a solute molecule across the membrane following the formation of a three-molecular complex carrier- Na^+ -solute. When the concentration of Na^+ ions is much higher than that of the solute it is assumed that the complex carrier- Na^+ ion is first formed followed by carrier- Na^+ -solute. In describing the formation of the three-molecular complex, the stage of Na^+ carrier association can be omitted. This reaction is included in the rate constant describing the association of the i -th solute with the preformed complex containing the carrier of the j -th transport system and Na^+ ions on both sides of the cell membrane

$$k_{ij}^1 = \text{Na}_1^+ k_{ij}^* \quad (1)$$

$$k_{ij}^{-2} = \text{Na}_2^+ k_{ij}^* \quad (2)$$

where Na_1^+ and Na_2^+ are concentrations of Na^+ ions outside and inside the cell,

respectively, and k_{ij}^* is the basic rate constant for the formation of the T_{ij} complex. The latter has the same value on both sides of the cell membrane. If the concentration of Na^+ ions outside the cell is several-fold higher than that inside the cell, the rate constants for the formation of the T_{ij} complex meet the condition

$$k_{ij}^1 \gg k_{ij}^{-2} \quad j = 1, \dots, r - 1 \tag{3}$$

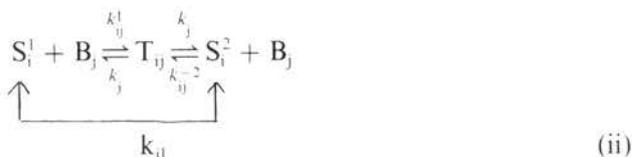
It is also assumed that systems $j = r, \dots, p - 1$ are asymmetric in terms of the rates of the complex formation. This is described by

$$k_{ij}^1 \gg k_{ij}^{-2} \quad j = r, \dots, p - 1 \tag{4}$$

Our next simplifying assumption is that both groups of the transport systems, i. e. $j = 1, \dots, p - 1$, are characterized by identical dissociation rates of the complex in the j -th transport system on both sides of the cell membrane, and for each solute, i. e.,

$$k_{ij}^{-1} = k_{ij}^2 = k_j \quad j = 1, \dots, p - 1 \tag{5}$$

For transport by third group systems it has been also assumed that the solute concentration is many times lower than the dissociation constant of the complexes formed in these systems, and that this dissociation constant is many times higher than that of complexes formed in the first two group systems. Under these assumptions, the third group of the transport systems may represent facilitated diffusion analogous to simple physical diffusion, and described by k_{il} . Based on the above assumptions, the transport described by relationship (i) takes the form shown by (ii)



$$i = 1, \dots, m; j = 1, \dots, p - 1; l = p, \dots, n$$

The first two groups of transport systems are described by the same equations; the above scheme does not differentiate between them. Keeping in mind that the solute concentration outside the cell remains effectively stable, system of differential rate equations describing the reaction can be written using scheme (ii). The same abbreviations as above are used for the species and their corresponding concentrations.

$$\frac{dB_j(t)}{dt} = -B_j(t) \sum_{i=1}^m (k_{ij}^1 S_i^1 + k_{ij}^{-2} S_i^2(t)) + 2k_j \sum_{i=1}^m T_{ij}(t) \quad (6)$$

$$\frac{dS_i^2(t)}{dt} = \sum_{j=1}^{p-1} (k_j T_{ij}(t) - B_j(t) k_{ij}^{-2} S_i^2(t)) + \sum_{l=p}^n k_{il} (S_i^1 - S_i^2(t)) \quad (7)$$

$$\frac{dT_{ij}(t)}{dt} = B_j(t) (k_{ij}^1 S_i^1 + k_{ij}^{-2} S_i^2(t)) - 2k_j T_{ij}(t) \quad (8)$$

It can be easily seen that the system (6—8) includes first integrals

$$B_j(t) + \sum_{i=1}^m T_{ij}(t) = B_j^0 \quad (9)$$

where B_j^0 is the total carrier concentration in the j -th system. Nevertheless, the dynamical system resulting from (6—8) and (9) is still complicated. In equilibrium for low molecular weight solutes such as amino acids or sugars the ratio S_i^2/S_i^1 does not exceed 10 (Anraku 1982). Thus, assuming $k_{ij}^1 \gg k_{ij}^{-2}$ (conditions (3) and (4)), $S_i^2 k_{ij}^{-2}$ may be omitted from equations (6—8). Hence, taking into account expression (9), equation (6) can be written as

$$\frac{dB_j(t)}{dt} = -B_j(t) \sum_{i=1}^m k_{ij}^1 S_i^1 + 2k_j (B_j^0 - B_j(t)) \quad (10)$$

The solution to this equation is

$$B_j(t) = B_j^0 \frac{\sum_{i=1}^m k_{ij}^1 S_i^1 \exp\left(-\left(\sum_{i=1}^m k_{ij}^1 S_i^1 + 2k_j\right)t\right) + 2k_j}{\sum_{i=1}^m k_{ij}^1 S_i^1 + 2k_j} \quad (11)$$

Substituting Eq. (11) into Eq. (8) we obtain

$$T_{ij}(t) = \frac{k_{ij}^1 S_i^1 B_j^0}{\sum_{i=1}^m k_{ij}^1 S_i^1 + 2k_j} \left(1 - \exp\left(-\left(\sum_{i=1}^m k_{ij}^1 S_i^1 + 2k_j\right)t\right)\right) \quad (12)$$

Taking into account (7), (12), and the above assumptions we get

$$S_i^2(t) = S_i^{02} \exp\left(-\sum_{l=p}^n k_{il} t\right) + S_i^1 \left(1 - \exp\left(-\sum_{l=p}^n k_{il} t\right)\right) + \sum_{j=1}^{p-1} \left[\frac{k_{ij}^1 k_j S_i^1 B_j^0 \left(\exp\left(-\left(\sum_{i=1}^m k_{ij}^1 S_i^1 + 2k_j\right)t\right) - \exp\left(-\sum_{l=p}^n k_{il} t\right)\right)}{\left(\sum_{i=1}^m k_{ij}^1 S_i^1 + 2k_j\right) \left(\sum_{i=1}^m k_{ij}^1 S_i^1 + 2k_j - \sum_{l=p}^n k_{il}\right)} \right] +$$

$$+ \left. \frac{k_{ij}^1 k_j S_i^1 B_j^0 \left(1 - \exp \left(- \sum_{l=p}^n k_{il} t \right) \right) \right]}{\sum_{l=p}^n k_{il} \left(\sum_{i=1}^m k_{ij}^1 S_i^1 + 2k_j \right)} \right] \quad (13)$$

Relationship (13) describes the concentration of the *i*-th solute inside the cell as a function of both its initial intracellular and extracellular concentration; it also describes the concentrations of other solutes and their affinities to the transport systems. S_i^{02} is the initial concentration of the *i*-th solute inside the cell.

By solving equations (6–8) under the assumption of constant concentrations of all the carrier forms over time, we obtain a simplified solution to the model:

$$S_i^{2u}(t) = S_i^{02} \exp \left(- \sum_{l=p}^n k_{il} t \right) + \left(\sum_{l=p}^n k_{il} S_i^1 + \sum_{j=1}^{p-1} \frac{k_{ij}^1 k_j S_i^1 B_j^0}{2k_j + \sum_{i=1}^m k_{ij}^1 S_i^1} \right) \left(\frac{1 - \exp \left(- \sum_{l=p}^n k_{il} t \right)}{\sum_{l=p}^n k_{il}} \right) \quad (14)$$

Differentiating both sides of Eq. (14) with respect to time, we obtain

$$\frac{dS_i^{2u}(t)}{dt} = \sum_{l=p}^n k_{il} (S_i^1 - S_i^{02}) \exp \left(- \sum_{l=p}^n k_{il} t \right) + \sum_{j=1}^{p-1} \frac{k_{ij}^1 k_j S_i^1 B_j^0 \exp \left(- \sum_{l=p}^n k_{il} t \right)}{2k_j + \sum_{i=1}^m k_{ij}^1 S_i^1} \quad (15)$$

Note that Eq. (15) may be written in the form

$$V_i = \frac{dS_i^{2u}}{dt} = K_{Di} (S_i^1 - S_i^{02}) + \sum_{j=1}^{p-1} \frac{V_{\max ij} S_i^1}{\sum_{i=1}^m S_i^1 + K_{Mij}} \quad (16)$$

where V_i is the rate of concentration change of the *i*-th solute inside the cell;

$$V_{\max ij} = k_j B_j^0 \exp \left(- \sum_{l=p}^n k_{il} t \right) \quad (17)$$

is the maximal transport rate mediated by the *j*-th system;

$$K_{Mij} = \frac{2k_j}{k_{ij}^1} \quad (18)$$

is the Michaelis constant for the *j*-th system, and

$$K_{Di} = \sum_{l=p}^n k_{il} \exp\left(-\sum_{l=p}^n k_{il} t\right) \quad (19)$$

corresponds to the rate constant characteristic for transport by systems with facilitated diffusion, independent of Na^+ ions.

Note, that the formula (16) generalizes the classical three-parameter equation which is frequently used in the transport studies (Floud and Fahn 1981; Kontro and Oja 1978; Lussier et al. 1982; Rosenberg et al. 1980). It should also be noted that the entities K_{Di} and $V_{\max ij}$, usually treated as constants within the classical equation are in fact time-dependent.

Obviously when time tends to infinity, solution to the model describe the exchange equilibrium which reach all the transport systems present in the cell membrane. Then, the rate of changes in the solute concentrations in the cell approaches zero.

$$\lim_{t \rightarrow \infty} \frac{dS_i^2(t)}{dt} = \lim_{t \rightarrow \infty} \frac{dS_i^{2u}(t)}{dt} = 0 \quad (20)$$

In the limit $t \rightarrow \infty$, the intracellular solute concentrations given by the simplified and the exact solutions to the model are expressed by the same equations describing solute concentrations in cells under exchange equilibrium

$$\lim_{t \rightarrow \infty} S_i^{2u}(t) = \lim_{t \rightarrow \infty} S_i^2(t) = S_i^1 + \sum_{j=1}^{p-1} \frac{k_{ij}^1 k_j S_i^1 B_j^0}{\left(\sum_{i=1}^m k_{ij}^1 S_i^1 + 2k_j\right) \sum_{l=p}^n k_{il}} \quad (21)$$

From (21) it appears that during the operation of transport systems of all the groups in the cell membrane, the intracellular solute concentration exceeds that outside the cell. At the same time if the rate of changes in the intracellular solute concentration is zero, Eq. (15) shows that transport runs in both directions

$$\sum_{l=p}^n k_{il} (S_i^{02} - S_i^1) = \sum_{j=1}^{p-1} \frac{k_{ij}^1 k_j S_i^1 B_j^0}{2k_j + \sum_{i=1}^m k_{ij}^1 S_i^1} \quad (22)$$

The left side of Eq. (22) describes efflux of solutes by systems of the third group (facilitated diffusion) whereas the right side describes influx of solutes mediated by both the first and the second group of saturable systems. With the exact solution to the model, the situation is qualitatively the same, but considerably more intricate relationships are obtained.

Calculations

The concentrations of amino acid taken up by platelets was measured in three separate experiments. Mean square error was lower than 10%. In order to get a set of parameters describing the transport of the respective amino acids all experimental data obtained for each amino acid were normalized using the equation

$$N_k = \frac{\langle N_a \rangle}{\langle N \rangle} N_r \quad k = r \quad (23)$$

where $\langle N \rangle$ is the mean value calculated for a single experiment, $\langle N_a \rangle$ is the mean value calculated for all experiments for the same amino acid and for the same conditions, i.e. 1 min incubation at 37°C, concentration of amino acids 0.43 mmol/l; N_r stands for the actual value obtained from the experiment, and N_k is the normalized value corresponding to N_r . The normalized values were used for the calculations of rate constants and concentrations of the carrier protein. Based on experimental data it was assumed that amino acids are transported to blood platelets by:

1. a system which is saturable and depends on Na^+ ions,
2. an unsaturable system independent of Na^+ ions. Furthermore, it was assumed that the initial concentrations of amino acids inside the cell were zero. Then, we can put $m = 1$, $n = 2$, and $p = 2$.

The best fit of the experimental data with the theoretical calculations is obtained with

$$F = \sum_{k=1}^u (N_k - N(k_{ij}^1, k_j, k_{il}, B_j^0, S_i^1, t_i))^2 \quad (24)$$

where u are all experimental points obtained from experiments performed with the amino acid studied, N_k is the normalized value, and $N(k_{ij}^1, k_j, k_{il}, B_j^0, S_i^1, t_i)$ is the theoretical value of N_k .

The minimum value obtained by Eq. (24) was sought by the numeric gradient technique (Korn and Korn 1968). The correlation coefficient was calculated as the final test of fitting of experimental data with the theoretical curves.

The figures show curves drawn through experimental points on the basis of the mathematical model. The curves were constructed using both the exact and the simplified solutions.

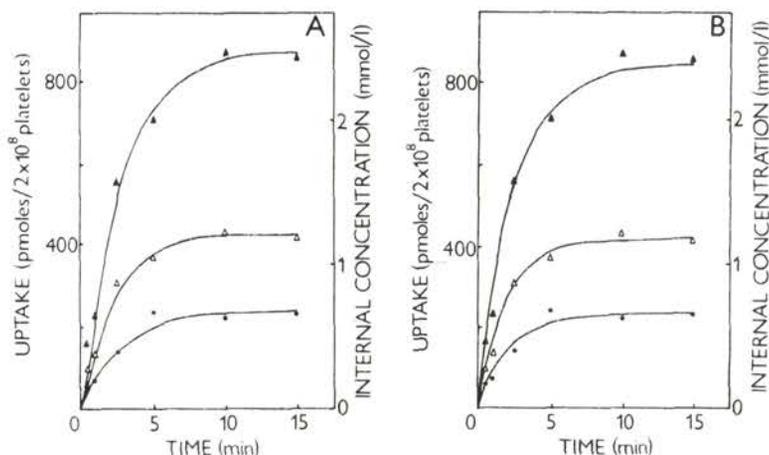


Fig. 1. Time dependence of transport of DL-Leu ($\Delta - \Delta$), L-His ($\blacktriangle - \blacktriangle$), and L-Glu ($\bullet - \bullet$), into human blood platelets. Curves were drawn by Eqs. (13) (panel A) and (14) (panel B). For curve parameters see Table I.

Results

Time dependences of the amino acid transport. The uptake of amino acids was measured during 15 min of incubation at 37°C. The concentration of amino acids was 0.43 mmol/l. The calculations were performed under the assumption that only L-leu was transported by a saturable system, and both D and L isomers of leucine were transported by nonsaturable systems. Satisfactory results can be obtained under this assumption only. Figure 1 shows time-dependences of the transport of amino acids. The curves were drawn according to the exact and the simplified solution to the model. Note, that both solutions are in good agreement with experimental data.

Concentration dependences. The uptake rates of His, Leu and Glu into human blood platelets over a concentration range of 0.1 to 15 mmol/l, and for 1 min of incubation are shown in Figure 2. The experimental data together with curves constructed by Eqs. (13) and (14) strongly suggest the presence of two components in the transport system i. e. a saturable and a nonsaturable. The values of V_{max} , K_M and K_D were calculated according to the simplified solution to the model (Eq. (16)) and are summarized in Table I.

Na⁺ ions-dependence of Leu, His and Glu uptake. In Na⁺-free buffer A, NaCl was replaced by equimolar amounts of choline chloride to give 140 mmol/l; the effect of increasing extracellular concentrations of Na⁺ ions on the uptake of

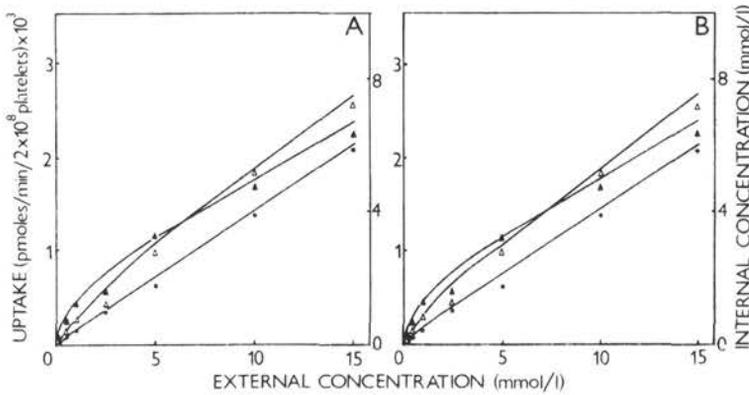


Fig. 2. Concentration dependence of DL-Leu ($\Delta - \Delta$), L-His ($\blacktriangle - \blacktriangle$), and L-Glu ($\bullet - \bullet$) uptake by human blood platelets. Curves in panels A and B were drawn by Eqs. (13) and (14), respectively. For curve parameters see Table I.

Table 1. Parameters describing transport of amino acids into blood platelets. They are given in: k_{11}^1 ($1 \text{ mmol}^{-1} \text{ s}^{-1}$), k_1 (s^{-1}), k_{12} (s^{-1}), B_1^0 ($\text{mmol} \cdot \text{l}^{-1}$), K_M ($\text{mmol} \cdot \text{l}^{-1}$), V_{\max} ($\text{pmol} \cdot \text{s}^{-1}$), and K_D ($\text{s}^{-1} \times 10^9$). V_{\max} and K_D were given in reference to 2×10^8 platelets. B is the estimated amount of the carrier protein per platelet. R is a correlation coefficient for experimental data and theoretical values calculated from the model.

	L-Leu	L-His	L-Glu
exact solution			
k_{11}^1	3.92×10^{-2}	4.20×10^{-2}	1.22×10^{-2}
k_1	8.92×10^{-3}	9.58×10^{-3}	2.93×10^{-3}
k_{12}	9.65×10^{-3}	7.18×10^{-3}	8.20×10^{-3}
B_1^0	2.512	3.142	1.368
R	0.987	0.989	0.982
simplified solution			
k_{11}^1	3.90×10^{-2}	4.19×10^{-2}	1.22×10^{-2}
k_1	8.90×10^{-3}	9.52×10^{-3}	2.92×10^{-3}
k_{12}	9.63×10^{-3}	7.45×10^{-3}	8.13×10^{-3}
B_1^0	2.502	3.116	1.362
B	2.7×10^4	3.3×10^4	1.5×10^4
K_D	2.59	2.12	2.28
K_M	0.456	0.454	0.479
V_{\max}	5.97	8.45	1.10
R	0.981	0.983	0.979

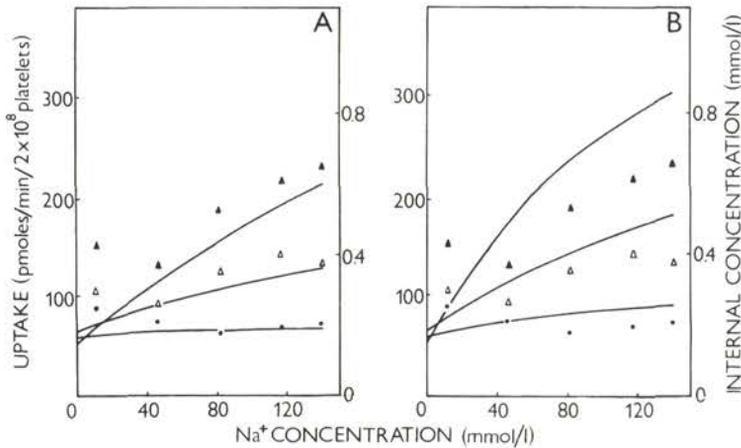


Fig. 3. Effect of the extracellular Na^+ concentration on the uptake rates of DL-Leu ($\Delta-\Delta$), L-His ($\blacktriangle-\blacktriangle$) and L-Glu ($\bullet-\bullet$). Curves in panels A and B were drawn by Eqs. (13) and (14), respectively. The condition $k_{11}^1 = k_{11}^*$ Na^+ was considered with $k_{11}^* = 3.0$; 2.8 ; $0.83 \times 10^{-4} \text{ mmol}^{-2} \text{ s}^{-1} \text{ l}^2$, for His, Leu and Glu, respectively

amino acids was analyzed in this medium (Fig. 3). The experiment was performed at 37°C , with 1 min incubation and $0.43 \text{ mmol/l } ^{14}\text{C}$ -amino acids.

The curves were drawn according to predictions of the model for parameters calculated from other experiments. The model calculations performed according to the exact solution (Eq. (13)) fitted relatively well the experimental data obtained at higher concentrations of Na^+ ions at the platelet outside (Fig. 3A). However, curves drawn on the basis of the simplified solution (Eq. (14)) were not consistent with the experimental points over the entire range of Na^+ concentrations (Fig. 3B).

In order to explain the totally different sensitivities of the transport system to extracellular Na^+ concentrations predicted by both the exact and the simplified solution, the time dependences of uptake rates were carefully analyzed. The exact and the simplified solution are illustrated in Figure 4 (the continuous and the dashed curve, respectively). After 1 min of incubation the amounts of amino acids taken up calculated by the model correspond to the fields under the curves, for period between 0 to 1 min. It is clear, that the simplified solution to the model does not describe the effect of Na^+ ions on the transport with sufficient accuracy. Indeed, in contrast to both the exact solution and experimental data, the simplified solution predicted excessive amounts of amino acids taken up. However, after prolonged incubation times both solutions give similar results.

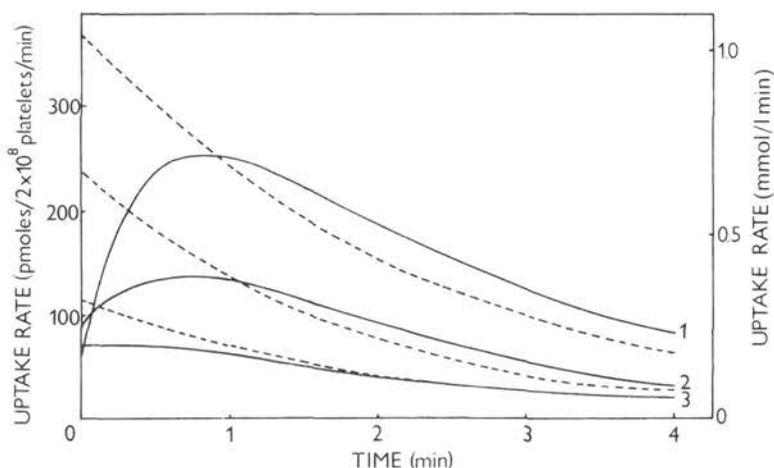


Fig. 4. Changes in the uptake rates of His (1), Leu (2), and Glu (3) during transport processes. The continuous and the dashed line corresponds to the exact and the simplified solution, respectively.

Table 2. The effect of metabolic inhibitors on transport of amino acids. Comparison of the effects of KCN and DNP on uptake of Leu, His and Glu by human blood platelets. Experimental data are shown together with values predicted by the model. The uptake of amino acids is expressed in pmoles per minute and 2×10^8 platelets. Preincubation time was 20 h.

Inhibitor (mmol/l)	DL-Leu		L-His		L-Glu	
	uptake	inhibition	uptake	inhibition	uptake	inhibition
DNP 5.0	67.3	48.2 %	127.9	44.6 %	65.0	8.5 %
DNP 2.5	70.9	45.4 %	136.8	40.8 %	66.2	6.8 %
KCN 5.0	49.2	62.2 %	63.3	72.6 %	60.2	15.2 %
KCN 2.5	90.9	30.1 %	126.7	45.2 %	63.6	10.4 %
Control	130.0	0.0 %	231.1	0.0 %	71.0	0.0 %
Theoretical values						
Eq. (13) 0.0	128.3	0.0 %	211.5	0.0 %	68.8	0.0 %
Eq. (13) max	66.8	47.9 %	53.2	74.8 %	59.1	14.1 %
Eq. (14) 0.0	181.6	0.0 %	301.1	0.0 %	90.2	0.0 %
Eq. (14) max	66.1	63.6 %	54.7	81.9 %	58.7	34.9 %

The effect of metabolic inhibitors of transport. In order to evaluate the energy requirement for Leu, His, and Glu uptake, experiments were performed in the presence of KCN and DNP. As shown in Table II both inhibitors reduced significantly the amounts of amino acids transported. It is assumed that prolon-

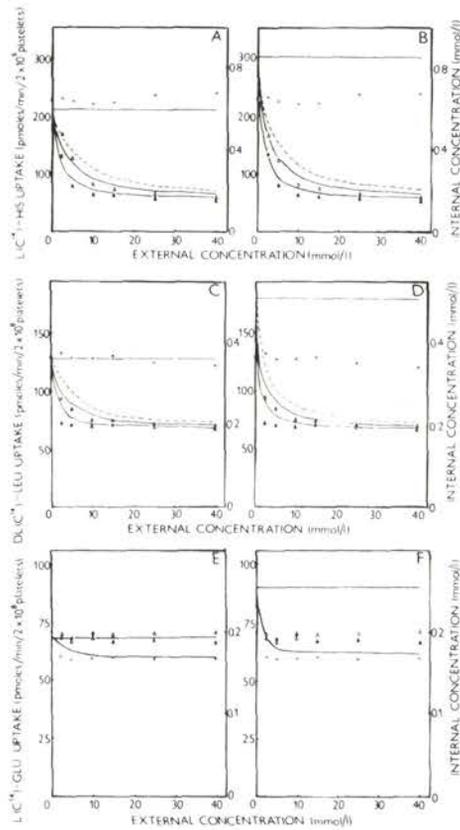


Fig. 5. Cross-inhibition of L(¹⁴C)-His (panels A and B), DL(¹⁴C)-Leu (panels C and D), and L(¹⁴C)-Glu (panels E and F) uptake by blood platelets. The curve in panels A, C, and E were drawn by the exact solution to the model. Increasing concentrations of Leu (Δ — Δ), His (\blacktriangle — \blacktriangle), and Glu (\bullet — \bullet) were introduced to incubation mixtures. The dashed line shows the predicted inhibition by Glu if it inhibited the fluxes of Leu and His. For curve parameters see Table I.

ged cell incubation with metabolic inhibitors results in the disappearance of Na^+ gradient and consequently the influx of amino acids is exclusively brought about by the linear transport component. The theoretical values were calculated by Eqs. (13) and (14).

Effects of other amino acids on the transport of His, Leu and Glu. The effects of other amino acids on the transport of Leu, His, and Glu were studied in the presence of competing amino acids in concentrations ranging between 0.5 and 40 mmol/l. The experiments were performed under conditions as above. Figure 5 shows mutual inhibition of the uptake of Leu, His, and Glu by human blood platelets. The curves were constructed by Eqs. (13) and (14) with $m = 2$, $n = 2$, and $p = 2$.

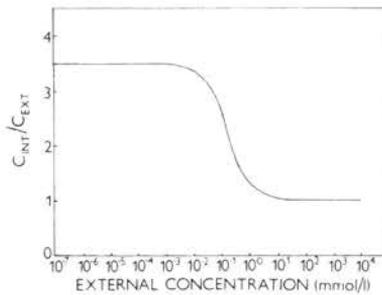


Fig. 6. The effect of the extracellular concentration of solutes on their accumulation inside the cell for exchange equilibrium. The curve was drawn by Eq. (21). Other parameters were the same as for histidine (Table 1).

Neither His nor Leu is displaced by Glu during their transport into blood platelets. The dashed lines in Figures 5AB and 5CD show the inhibition of fluxes of Leu and His by Glu, supposing that all of them are transported by the same system.

The rate constants and concentrations of the carrier protein calculated for Leu, His, and Glu are given in Table I. It is clear that Leu and His are transported to human blood platelets by the same saturable system while the system mediating the flux of glutamic acid functions independently. Only the constant rates describing the linear component of the transport are comparable for these amino acids.

All the experimental data obtained from these experiments show that even for extremely high concentrations of competing amino acids, the uptake of radiolabelled amino acids is not completely restrained. This is in good agreement with the model. For the same labelled and nonlabelled solute the rate constants of complex formation meet the condition

$$k_{11}^1 = k_{21}^1 \tag{25}$$

Transport inhibition of a radiolabelled solute by the same but cold solute is described by the equation

$$\begin{aligned} \frac{dS_1^{2*}(t)}{dt} &= k_{12}(S_1^{1*} - S_1^{02}) \exp(-k_{12}t) + \\ &+ \frac{k_1 S_1^{1*} B_1^0 [\exp(-k_{12}t) - \exp(-(k_{11}^1(S_1^{1*} + S_1^1) + 2k_1)t)]}{S_1^{1*} + S_1^1 + \frac{2k_1 - k_{12}}{k_{11}^1}} \end{aligned} \tag{26}$$

where S_1^{1*} and S_1^{2*} are the concentrations of the radiolabelled solute outside and inside the cell, respectively. For a simplified solution we have

$$\frac{dS_1^{2u*}(t)}{dt} = \left[k_{12}(S_1^{1*} - S_1^{02}) + \frac{k_1 S_1^{1*} B_1^0}{S_1^{1*} + S_1^1 + \frac{2k_1}{k_{11}}} \right] \exp(-k_{12}t) \quad (27)$$

Note, that the transport rate is not zero, even if the extracellular concentration of the cold solute tends to infinity.

The theoretical predictions of the model. Figure 6 illustrates the ratio of the intracellular to extracellular solute concentration as a function of the extracellular concentration for equilibrium exchange, as predicted by the model. At low extracellular solute concentrations, the accumulation inside the cell is very efficient and the transport is mainly mediated by the system exhibiting high affinity to the solute. At higher extracellular concentrations, the solute is predominantly transported by the low affinity diffusion system.

The amounts of the carrier molecules were estimated by model calculations. The calculations were done under the assumption that all carrier molecules can form complexes with amino acids present close to the membrane. The volume of the close-to-membrane sphere is assumed to be about 1 % of the cell volume. The theoretical values are given in Table I.

Discussion

In order to overcome some limitations inherent to steady-state techniques (which are commonly applied to the study of transport phenomena) we attempted to characterize fluxes of solutes across the cell membrane in pre-steady states. In contrast to other models our model describes the transport in general with a multiplicity of different systems being simultaneously operative in the cell membrane and with different solutes present in the environment.

Description of transport at exchange equilibrium using classical carrier or channel models for more than one system operative at a time leads to misinterpretations of experimental data. Exchange equilibrium may be reached by several systems, not by a particular one, and each system during a total exchange equilibrium may be far from its own exchange equilibrium.

Our model takes into account the effect of extracellular Na^+ concentration on the flux rates of solutes, and the inhibitory effect on the carrier mediated transport of certain toxic substances, such as dinitrophenol or cyanide.

Each saturable transport system is characterized by three separate parameters, i. e. rate constants of the formation and dissociation of carrier-solute

complexes, and by the total concentration of the carrier protein of the given transport system. These parameters determine V_{max} , the maximal transport velocity, and K_M , the constant corresponding to the Michaelis constant.

Our model considers changes in the solute concentration, and those in the concentrations of the carrier protein, both free and solute associated, occurring during the transport. Owing to this, it allows a prediction of the course of transport at a given time based on initial concentrations of the solute outside and inside the cell. Such a prediction would be of importance for our understanding of how drugs and other bioactive substances are transported across the cellular membranes.

According to the model, transport systems with low affinities to the solute and independent of Na^+ ions are described by a single parameter, directly connected with the constant characteristic for the linear transport component (Eq. 19).

The model introduced (Eq. (16)) is aimed at generalizing the three-parameter equation which is frequently used in the study of transport phenomena (Floud and Fahn 1981; Kontro and Oja 1978; Lussier et al. 1982; Oja and Vahvelainen 1975; Rosenberg et al. 1980).

Experimental data interpreted by the model calculations unequivocally show that there are two systems involved in the transport of Leu, His, and Glu. The first one is saturable and specific for the solutes transported. Its operation depends upon Na^+ gradient and is associated with metabolic processes of platelets. The other system is unsaturable and corresponds to facilitated diffusion.

There is evidence that Leu and His are transported to blood platelets by the same saturable system. Almost identical parameters describing the fluxes of both amino acids as well as cross-inhibition experiments presented in this study strongly support this idea. This is consistent with observations in other mammalian cells (Cierniewski and Walkowiak 1983; Oxender et al. 1977; Shotwell et al. 1981) but it contradicts the results published by Yardimci et al. (1981a, b). The latter authors have suggested that Leu and His are transported to blood platelets independently, though they found and partially purified carrier proteins for both amino acids with similar chromatographic properties (Yardimci et al. 1981b).

In agreement with other reports (Yardimci et al. 1981a, b; Zieve and Solomon 1968), our study suggests that the flux of Glu is not inhibited by Leu or His.

The amount of the carrier molecules, involved in the transport of Leu and His by the saturable system per platelet, as estimated by model calculations, was similar ($2.7\text{--}3.3 \times 10^4$, Table I). Lower quantities of the carrier protein were found for Glu (1.5×10^4). These values are of the same order as those reported for other platelet receptors, e.g. for ADP or fibrinogen. For the high affinity

ADP receptor, values of $2-6 \times 10^4$ (Lips et al. 1980), and for the fibrinogen receptor $4-6 \times 10^4$ (Walkowiak et al. 1987) were reported. However, it must be pointed out that owing to possible errors in estimating the volume in which the carrier interacts with amino acids, these values may not be correct. Nevertheless, the calculations performed in this work show that, the different carriers for different amino acids are present in similar quantities in blood platelet membranes.

Certainly, the model presented is not limited to the case of the transport of amino acids. It seems to provide a useful tool for the study of other transport processes across biological membranes.

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