Thyroid hormone concentrative uptake in rat erythrocytes

Involvement of the tryptophan transport system T in countertransport of tri-iodothyronine and aromatic amino acids

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The kinetic properties of transport system T, which is specific for uptake of aromatic amino acids, were studied in rat erythrocytes in the presence of leucine in order to block the neutral amino acid transport system L. Since the tri-iodothyronine (T_3) transport system and system T are closely related, the *trans* effect of T_3 and tryptophan on [3H]tryptophan transport and the *trans* effects of aromatic amino acids on [^{125}I] T_3 transport were studied. Equilibrium-exchange, zero-*trans* and infinite-*trans* studies of [3H]tryptophan transport indicated that system T in rat erythrocytes is a simple carrier with exchanging properties resulting in *trans*-acceleration of influx and *trans*-inhibition of efflux when tryptophan was present at the *trans* side of the membrane. In erythrocytes preloaded with unlabelled tryptophan, countertransport resulted in a 7-fold accumulation of labelled substrate inside the cells. T_3 on the *trans* side of the membrane inhibited both influx and efflux of tryptophan, with K_i values similar to the K_m values of the T_3 transport system. Extracellular tryptophan *trans*-inhibited [^{125}I] T_3 efflux in a manner similar to [3H]tryptophan efflux. Preloading erythrocytes with tryptophan resulted in *trans*-acceleration of T_3 uptake and a transient 5-fold accumulation of free T_3 into erythrocytes. Phenylalanine and tyrosine (but not the D-isomer of tryptophan or non-aromatic amino acids) also produced *trans*-acceleration for T_3 uptake and T_3 countertransport. These results are compatible with a kinetic model assuming a common simple carrier of T_3 and tryptophan transport and point to a countertransport pathway driving the uphill uptake of T_3 by hetero-exchange with intracellular aromatic amino acids.

INTRODUCTION

Thyroxine is deiodinated in peripheral tissues to active tri-iodothyronine (T_3) which binds to chromatin-associated receptors and subsequently regulates the expression of specific genes in target cells [1]. Thyroid hormones have first to cross the plasma membrane to reach their intracellular targets, and there is increasing evidence that this is performed by specialized, saturable and stereospecific transport systems [2–6].

Several studies *in vivo* point to the key role of these transport systems in thyroid hormone action, and suggest that active energy-dependent processes are involved in the cellular uptake of thyroid hormones [7–10]. Also, a number of studies *in vitro* on isolated tissues or cells suggest Na^+ - and ATP-dependent thyroid hormone transport across the plasma membrane [11–14]. Most of the studies have dealt with the inhibitory effect of metabolic blockers (such as KCN, oligomycin or antimycin) on thyroid hormone uptake by target cells [2–5,12,15].

However, other studies indicate that carrier-mediated T_3 uptake appears to be Na^+ -independent in several cell types [6,16–19]. Also, various drugs, usually used as metabolic blockers, were either without effect or inhibited the T_3 transport systems independently of ATP depletion [6,17,19,20]. These observations are compatible with facilitated rather than active transport, suggesting that mechanisms other than direct coupling to ionic gradients and ATP hydrolysis may be involved in active thyroid hormone uptake.

One way the cell can maintain a gradient of a transported substance is by hetero-exchange with intracellular substrates sharing the same carrier. Thus the Na^+ -independent amino acid

transport system L (which has strongly exchanging properties) can drive the uphill transport of neutral amino acids [21]. As thyroid hormones are iodoamino acids, they may be taken up by such a mechanism, which would imply sharing transport systems with other amino acids.

There is evidence that thyroxine is not transported in Ehrlich cells by neutral amino acid carriers [22] and that the T_3 uptake mechanism in hepatoma cells is distinct from the amino acid transport system A [16]. However, in embryonic-chick cartilage and isolated rat thymocytes, thyroid hormones appear to enhance amino acid uptake preferentially via the Na^+ -independent system L rather than via the Na^+ -dependent system A (reviewed in [23]). On the other hand, recent reports indicate that phenylalanine and other L-system amino acids inhibit the internalization of thyroid hormones in neuroblastoma cells [24]. We previously showed [25] that the T_3 transport system of rat erythrocytes was related to amino acid transport system T, previously characterized in human erythrocytes [26,27] as a Na^+ -independent system, specific for aromatic amino acids. T_3 and tryptophan are taken up into erythrocytes by interacting pathways whose activities are linked in the erythrocytes of several species [25]. In particular, both activities are considerably higher (100–500 times) in rat erythrocytes than in human erythrocytes.

None of these studies considered countertransport, i.e. stimulation of thyroid hormone uptake by intracellular amino acids. The present work was therefore undertaken to study the detailed kinetic properties of system T in rat erythrocytes (previously used as a cellular model to characterize the kinetics of T_3 transport [18,25,28]) and its exchanging properties, particularly in relation to T_3 countertransport.

Abbreviations used: T_3 , 3,3',5-tri-iodo-L-thyronine.

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EXPERIMENTAL

Chemicals

L-[3'- 125 I]T₃ (sp. radioactivity 3 mCi/ μ g) and L-[5- 3 H]tryptophan (sp. radioactivity 30 Ci/mmol) were from Amersham (U.K.). Unlabelled T₃ and amino acids were from Sigma. Plastic tubes and pipette tips were silicone-treated (Sigmacote, Sigma).

Preparation of washed erythrocytes

Blood from male Wistar rats (250 g; Iffa Credo, France) was collected into heparin, and erythrocytes were sedimented and washed as previously described [18,28] and suspended in buffer A (125 mM-NaCl, 20 mM-KCl, 4 mM-MgCl₂, 10 mM-glucose, 4.05 mM-Na₂HPO₄, 0.95 mM-NaH₂PO₄, pH 7.4). Washed erythrocytes were used immediately after preparation. Cell concentrations were determined with a Coulter counter.

Measurement of T₃ and tryptophan fluxes

Both cell preloading with T₃ or amino acids and the transport experiments were performed at 25 °C in buffer A containing 20 mM-leucine. The time courses of influx or efflux of [3 H]tryptophan (8×10^5 c.p.m./ml; 5 μ M) and of [125 I]T₃ (6×10^4 c.p.m./ml; 0.2 nM) were studied with 8×10^8 cells/ml and 10⁸ cells/ml respectively.

The kinetic constants of [3 H]tryptophan influx or efflux (zero-*trans*, equilibrium-exchange and T₃-*trans*-inhibition) were determined under initial-velocity conditions, as described previously for [125 I]T₃ transport [28], except that the erythrocyte concentration was 8×10^8 cells/ml, preloading was for 15 min at 25 °C [experiments with labelled (8×10^5 c.p.m./ml) or unlabelled tryptophan] or 2 h at 25 °C (experiments with unlabelled T₃), and 20 mM-leucine was present during preloading and transport. Initial velocities of influx and efflux were measured over the first 5 s. These values were subtracted from the zero-time values (efflux experiments), or the zero-time values were subtracted from them (influx experiments). All assays were performed in triplicate. Infinite-*trans* influx of [3 H]tryptophan was performed as zero-*trans* influx, except for a 2 h preincubation step of the cells at 25 °C in the presence of 10 mM unlabelled tryptophan.

The *trans* effects (*trans*-acceleration or *trans*-inhibition) of a saturating concentration of tryptophan (5 or 10 mM; see the Figure legends) on [125 I]T₃ (0.2 nM) influx or [3 H]tryptophan (5 or 10 μ M) influx or efflux were evaluated as follows: cell-associated radioactivity was determined (in triplicate) after 0, 2, 3, 4 and 5 s of influx or efflux of labelled substrates in the presence or absence of tryptophan at the *trans* side of the membrane, and the initial velocities were determined by linear regression (cell-associated radioactivity versus time).

Influx and efflux were terminated as described in [28] by adding an ice-cold solution of unlabelled T₃ in buffer A (10 μ M final concn.). The cells were washed with buffer A [25,28] and the radioactivity of the cell pellets was determined in a γ -radiation counter, for [125 I]T₃, or by liquid scintillation, after solubilization and deproteinization, for [3 H]tryptophan [25].

The equilibrium concentrations of labelled and unlabelled tryptophan and unlabelled T₃ were determined as previously described [28]. Free T₃ concentrations were corrected for instantaneous adsorption at the cell surface [25].

RESULTS AND DISCUSSION

Kinetics of tryptophan transport by system T in rat erythrocytes

System T in rat erythrocytes was characterized by using the formalism developed by Lieb & Stein [29,30], in the presence of 20 mM-leucine to exclude tryptophan transport via system L

[25,27]. The outside of the erythrocytes was arbitrarily designated side 1 and the cytoplasmic side as side 2. Maximal velocities (V) and apparent Michaelis-Menten constants (K) were determined in the three transport modes: zero-*trans* influx (zt,12), zero-*trans* efflux (zt,21) and equilibrium-exchange (ee). Zero-*trans* influx and efflux of tryptophan through system T were saturable and obeyed simple Michaelis-Menten kinetics (Fig. 1). The mean K values for influx (K_{12}^{zt}) and efflux (K_{21}^{zt}) obtained from three independent experiments were 0.47 and 0.99 mM respectively (Table 1). The V was lower for influx than for efflux. The asymmetry parameter calculated from the K ratio ($A = K_{12}^{zt}/K_{21}^{zt}$) was 0.47, similar to the value of 0.46 calculated from the V ratio ($A = V_{12}^{zt}/V_{21}^{zt}$). Hence the apparent affinity is ~ 2 -fold higher outside than inside the cells, whereas the reverse holds for maximal velocities of transport. This asymmetry of the tryptophan transport system is opposite to that of the related T₃ carrier, whose affinity for T₃ is ~ 6.5 times higher inside than outside the erythrocyte [28].

Substrate concentrations were equal on both sides of the membrane in equilibrium-exchange experiments. Under this condition, K and V (K^{ee} and V^{ee}) were ~ 1.3 times the respective values for zero-*trans* influx and 1.5–1.6-fold lower than the respective values for zero-*trans* efflux (Table 1).

The resistance parameters R_{12} , R_{21} and R_{ee} are the reciprocals of the maximum velocities V_{12}^{zt} , V_{21}^{zt} and V^{ee} , and are functions of the rate constants for conformational changes of the simple carrier [30] in one of the three modes: (1) loaded in the 1-to-2 (outside to inside) direction and empty in the 2-to-1 direction (zero-*trans* influx); (2) loaded in the 2-to-1 direction and empty in the 1-to-2 direction (zero-*trans* efflux); and (3) loaded in both directions (equilibrium-exchange). R_{00} , which is equal to $R_{12} + R_{21} - R_{ee}$ [30], is the resistance parameter when the carrier is empty in both directions. As shown in Table 1, a non-zero value of R_{00} can be calculated from the experimental values of the resistance parameters. This eliminates a pore model for transport of tryptophan, but is compatible with a simple carrier model according to the formalism developed by Lieb & Stein [29,30]. The simple carrier model assumes two interconvertible conformations of the unloaded carrier, each of which interacts with substrate at one surface of the membrane [30,31].

Fig. 1. Hanes plot (s/v versus s) of tryptophan transport by rat erythrocytes

Initial velocities (v) of [3 H]tryptophan transport were assayed (see the Experimental section) at tryptophan concentrations (s) of 0.1–2 mM, with erythrocytes either not preincubated (zero-*trans* influx; $\circ$) or pre-equilibrated with 10 mM unlabelled tryptophan (infinite-*trans* influx; $\blacksquare$), unlabelled tryptophan at concentrations identical with the extracellular concentrations used for uptake (equilibrium-exchange influx; $\triangle$), or [3 H]tryptophan (zero-*trans* efflux; $\square$). Values are means of triplicates (coefficient of variation was $< 10\%$ and $< 15\%$ in influx and efflux experiments respectively). Kinetic constants were obtained by linear regression. The abscissa intercepts of the lines correspond to $-K$, the ordinate intercepts to K/V and the slopes to $1/V$.

Table 1. Kinetic parameters of tryptophan transport in rat erythrocytes

The kinetic parameters K (Michaelis constant), V (maximal velocity) and Π (first-order rate constant: V/K) were obtained from Hanes plots by linear regression as described in the legend to Fig. 1. Results are means $\pm$ S.D. for n independent experiments.

			Experimental values ($\pm$ S.D.)		
Procedure	Direction	n	V ($\mu\text{mol}/\text{min}$ per 8×10^{10} cells)	K (mM)	Π (min^{-1})
Zero-trans	Out $\Rightarrow$ in	3	$V_{12}^{\text{zt}} = 12 \pm 1$	$K_{12}^{\text{zt}} = 0.47 \pm 0.03$	0.025
	In $\Rightarrow$ out	3	$V_{21}^{\text{zt}} = 26 \pm 8$	$K_{21}^{\text{zt}} = 0.99 \pm 0.12$	0.026
Equilibrium-exchange	Out $\Rightarrow$ in	5	$V^{\text{ee}} = 16 \pm 3$	$K^{\text{ee}} = 0.62 \pm 0.06$	0.026
Derived resistance parameters ($\pm$ S.D.)			(min/mM)		
$R_{12} = 1/V_{12}^{\text{zt}}$			85 ± 6		
$R_{21} = 1/V_{21}^{\text{zt}}$			43 ± 2		
$R_{\text{ee}} = 1/V^{\text{ee}}$			64 ± 1		
$R_{00} = R_{12} + R_{21} - R_{\text{ee}}$			64 ± 4		
Independent estimates of affinity parameter K^{00}					
Estimate using			Value (mM)		
$K^{00} = K_{12}^{\text{zt}} (R_{12}/R_{00})$			0.62		
$K^{00} = K_{21}^{\text{zt}} (R_{21}/R_{00})$			0.66		
$K^{00} = K_{\text{ee}} (R_{\text{ee}}/R_{00})$			0.62		
			mean = 0.63 ± 0.03		

The three estimates of K^{00} , the simple carrier affinity parameter, were similar (Table 1), with a mean value of 0.63 ± 0.03 mM. This also indicates that the simple carrier model holds [31] for tryptophan transport in rat erythrocytes.

The ratio R_{00}/R^{ee} is approx. 1 (Table 1), so that the mobility (in both directions) of the empty carrier is equal to that of the loaded carrier. However, the fact that equilibrium-exchange V is lower than zero-trans efflux V , but higher than zero-trans influx V , suggests that the empty carrier has a higher mobility than the substrate-loaded carrier in the 1-to-2 direction, whereas the reverse would hold for the 2-to-1 direction.

Finally, the first-order rate constants for exchange ($\Pi^{\text{ee}} = V^{\text{ee}}/K^{\text{ee}}$) and for the two zero-trans fluxes ($\Pi_{12}^{\text{zt}} = V_{12}^{\text{zt}}/K_{12}^{\text{zt}}$ and $\Pi_{21}^{\text{zt}} = V_{21}^{\text{zt}}/K_{21}^{\text{zt}}$) are similar (Table 1), as expected for a simple carrier [31]. This demonstrates the internal consistency of the data and the methods used to determine the kinetic parameters.

Effects of extracellular tryptophan or T_3 on tryptophan efflux

Fig. 2 shows the time course of labelled tryptophan efflux from preloaded erythrocytes. A saturating concentration (5 mM) of unlabelled tryptophan in the efflux medium slowed the initial phase of efflux. A new equilibrium level was reached which was identical with the control level with no unlabelled tryptophan (Fig. 2a). Indeed, the higher rate of inside re-orientation of empty carrier than of loaded carrier, predicted from the kinetic data (Table 1), results in *trans*-inhibition of efflux by extracellular substrate.

In the experiment shown in Fig. 2(b), the initial velocities of efflux (0–5 s) were obtained by linear regression, as described in the Experimental section. The slope determined in the presence of 5 mM unlabelled tryptophan ($3.80 \pm 0.15 \text{ min}^{-1}$) was significantly lower ($P < 0.01$; F test) than that measured in the

absence of tryptophan ($5.43 \pm 0.09 \text{ min}^{-1}$). [^3H]Tryptophan efflux was *trans*-inhibited 1.5-fold (mean of two independent determinations; range 1.4–1.6). This value is close to the ratio of the measured V for zero-trans efflux and equilibrium-exchange, as expected for the carrier model [32], since $V_{21}^{\text{zt}}/V^{\text{ee}} \approx 1.6$. *Trans*-inhibition of system-T-mediated tryptophan efflux also occurs in human erythrocytes [27].

The effect of extracellular T_3 on tryptophan efflux was also investigated (Figs. 2a and 2b): efflux was completely *trans*-inhibited by $10 \mu\text{M}$ T_3 . This is similar to the previously reported effect of T_3 on its own transport system, for which *trans*-inhibition occurs in both the inward and outward directions [28]. This observation validates the use of $10 \mu\text{M}$ unlabelled T_3 as a stop solution for blocking both influx [25] and efflux (the present work) of labelled tryptophan and as a wash solution for preventing the loss of internalized tryptophan.

This inhibition was studied at various concentrations of extracellular unlabelled T_3 by using Dixon plots of the data (Fig. 3a). Since the concentration of [^3H]tryptophan was $\ll K_{21}^{\text{zt}}$, the *trans*-inhibition constant (*trans*- K_i) of outside T_3 was obtained from the abscissa intercept of the plot. The mean value of the constant from three independent experiments was $0.14 \pm 0.03 \mu\text{M}$. This *trans*- K_i value is similar to the K of T_3 uptake ($0.13 \mu\text{M}$) and to the *trans*- K_i of T_3 efflux ($0.12 \mu\text{M}$) reported previously [28], suggesting that *trans*-inhibition of tryptophan efflux by T_3 is mediated by the T_3 transport system.

Trans-inhibition of T_3 efflux by extracellular tryptophan

The efflux of labelled T_3 from preloaded erythrocytes was slower when the efflux medium contained 5 mM unlabelled tryptophan (Fig. 4). The initial velocity of [^{125}I] T_3 efflux (linear-regression slope of cell-associated radioactivity versus time, as

Fig. 2. Time course of tryptophan efflux from preloaded erythrocytes

Cells were preincubated with 5 μM-[³H]tryptophan for 15 min at 25 °C, centrifuged and resuspended in buffer A (●), or in buffer A containing 5 mM unlabeled tryptophan (○), or in buffer A containing 10 μM unlabeled T₃ (△). Efflux was stopped at timed intervals (0–90 s, a; 0–5 s, b) and the remaining cell-associated radioactivity was determined in duplicate (a) or triplicate (b). Results are expressed as percentages of the zero-time values.

Fig. 3. Dixon plots (1/v versus inhibitor concn.) of *trans*-inhibition by T₃ of tryptophan transport

(a) Erythrocytes were preloaded with [³H]tryptophan (5 μM, 2 h at 25 °C), centrifuged, resuspended in buffer A containing increasing concentrations of unlabeled T₃ (0–200 nM), and initial velocities (*v*) of tryptophan efflux were determined in triplicate. (b) Erythrocytes were preincubated in buffer containing increasing concentrations of unlabeled T₃ (0–600 nM, 15 min at 25 °C), centrifuged, resuspended in buffer A containing [³H]tryptophan (10 μM) and initial velocities (*v*) of tryptophan influx were determined in triplicate. Equilibrium concentrations of free T₃ were determined, as reported in the Experimental section, in parallel preincubations containing the same concentrations of unlabeled T₃ and a tracer amount (25 pM) of [¹²⁵I]T₃.

described in the Experimental section) was decreased 1.4–1.5-fold (two independent experiments) as determined under initial-velocity conditions. This is very similar to the effect on labelled tryptophan efflux, suggesting that the *trans*-inhibition of T₃ efflux

Fig. 4. Time course of T₃ efflux from preloaded erythrocytes

Cells were preincubated with 0.2 nM-[¹²⁵I]T₃ for 15 min at 25 °C, centrifuged, and efflux was initiated by resuspension in buffer A with (●) or without (○) 5 mM unlabeled tryptophan. Cell-associated radioactivity (duplicate assays) was determined and expressed as the percentage of the zero-time value.

Fig. 5. Time courses of tryptophan and T₃ uptakes in amino acid-preloaded erythrocytes

Cells were preincubated in the absence (control) or presence of various amino acids (10 mM) or in the presence of T₃ (10 μM) for 2 h at 25 °C, centrifuged and resuspended in uptake buffer containing labelled substrates. (a) Cells were preloaded with tryptophan (○) or T₃ (▲) or were unloaded (●), and [³H]tryptophan (5 μM) uptake was monitored as a function of time (left ordinate). In a parallel experiment, cells were preloaded in the presence of 10 mM-tryptophan and a tracer amount (30 nM) of [³H]tryptophan. Cells were centrifuged and tryptophan efflux was monitored (■) as described in the legend to Fig. 2, and expressed as the percentage of the zero-time value (right ordinate). (b) Cells were preloaded with tryptophan (○), tyrosine (△), phenylalanine (◇) or D-tryptophan (□). Results of preloading with serine or preincubation with no amino acid were indistinguishable (●). Uptake of [¹²⁵I]T₃ (0.2 nM) was monitored as a function of time. Values (panels a and b) are means of duplicate assays.

is also related to the relative translocation rates of the empty and loaded tryptophan carriers.

Kinetics of tryptophan influx in the presence of intracellular tryptophan or T₃

Fig. 5(a) illustrates the effect of preloading the cells with a saturating concentration of unlabeled tryptophan (10 mM) on the time course of labelled tryptophan uptake. Intracellular tryptophan accelerated the influx of [³H]tryptophan and caused its transient accumulation against the concentration gradient to about 7 times the equilibrium control level after a 2 min uptake

(mean of two experiments). The efflux of 10 mM preloaded tryptophan (together with a tracer amount of [^3H]tryptophan) was monitored in an independent experiment (Fig. 5a) and found to be half-maximal within approx. 3 min. Therefore, in the countertransport experiment, intracellularly accumulated [^3H]tryptophan returned to the control level as a consequence of the time-dependent loss of preloaded unlabelled tryptophan. This *trans*-acceleration of influx is in agreement with a higher rate of external translocation of loaded carrier than of empty carrier, in accord with the kinetic data (R_{12} being higher than R_{ee}) (Table 1).

Trans-acceleration of [^3H]tryptophan influx was studied under initial-velocity conditions in the presence of preloaded tryptophan (10 mM). Initial velocities were calculated from the slopes obtained by linear regression (cell-associated radioactivity versus time), as described in the Experimental section. The ratio of the slopes, relative to control, was 1.5 ± 0.1 (mean of three independent experiments), close to the ratio of the experimental V values for zero-*trans* influx and equilibrium exchange (Table 1), since $V^{ee}/V_{12}^t = 1.3$. Again, this correlates well with the carrier model [32].

The kinetic parameters K and V can be determined under infinite-*trans* conditions (K^{it} and V^{it}) (i.e. in the presence of a saturating concentration of unlabelled substrate on the *trans* side of the membrane) provided that $R_{00} \neq 0$ [31]. A Hanes plot of uptake data obtained in the presence of a saturating intracellular concentration of tryptophan (infinite-*trans* influx) is shown in Fig. 1. The mean values from three independent experiments were $K_{12}^{\text{it}} = 0.45 \pm 0.04$ mM and $V_{12}^{\text{it}} = 21 \pm 7$ $\mu\text{mol}/\text{min}$ per 8×10^{10} cells. Comparison with the zero-*trans* influx values (Table 1) indicates that V , rather than K , is affected by substrate on the *trans* side of the membrane. These experiments provided an additional independent estimate of the simple carrier affinity parameter, $K^{00} = K_{12}^{\text{it}} (R_{ee}/R_{21})$ [31]. The resulting value was 0.67 mM, compatible with the mean K^{00} value in Table 1 and therefore with the simple carrier model.

Finally, a high concentration of T_3 inside the cells (3.5 μM free T_3) completely inhibits tryptophan uptake (Fig. 5a). The *trans*-inhibition of labelled tryptophan influx was studied with erythrocytes preloaded with increasing concentrations of unlabelled T_3 (Fig. 3b). The mean inside *trans*-inhibition constant from three independent experiments was 26 ± 5 nM. This K_i value is similar to the K of T_3 efflux (18 nM) and to the *trans*- K_i of T_3 influx (21 nM) reported previously [28]. This again strongly suggests that interaction of T_3 with tryptophan transport is mediated by the previously characterized T_3 carrier.

Countertransport of aromatic amino acids and T_3

The uptake of [^{125}I] T_3 was accelerated when erythrocytes were preloaded with unlabelled tryptophan (10 mM), and the hormone accumulated above the equilibrium level obtained in the absence of tryptophan (Fig. 5b). The overshoot was similar to that observed for [^3H]tryptophan uptake (2.9 ± 0.2 times the control, $n = 5$). As previously reported [18], based on the intracellular water content of erythrocytes, T_3 is accumulated about 70-fold over the medium concentration at equilibrium in the unloaded control condition. This was attributed to intracellular binding of T_3 [18], free hormone being in equilibrium across the membrane. In contrast, T_3 is accumulated about 360-fold over the medium concentration in tryptophan-loaded erythrocytes, at maximum overshoot. Assuming that the intracellular binding (trapping) system is not significantly saturated in this range of T_3 concentrations, this indicates that the concentration gradient of free T_3 across the membrane (free intracellular T_3 /free extracellular T_3) can reach a value of 5.2 ± 0.8 ($n = 5$) in the presence of intracellular tryptophan.

Trans-inhibition, rather than *trans*-acceleration and overshoot, occurred when cells were preloaded with D-tryptophan instead of L-tryptophan (Fig. 5b). D-Tryptophan, although it is a competitor of L-tryptophan uptake [25,27], is said not to be transported by system T [27]. This indicates that efflux via system T is required for acceleration and subsequent overshoot, and not just binding to it. Preloading with 10 mM-serine, a substrate of the ASC amino acid-transport system, had no effect on the time course of T_3 uptake.

Preloading with phenylalanine, another substrate of system T, resulted in a stronger *trans*-acceleration of [^{125}I] T_3 initial uptake (Fig. 5b), suggesting that the outward movement of the phenylalanine-carrier complex is faster than that of the tryptophan-carrier complex. However, the overshoot was more transient and reached a lower level than with preloading (after a 25 s uptake, the concentration gradient of free T_3 was ~ 1.5 ; mean of two experiments), possibly owing to the phenylalanine efflux being more rapid than the tryptophan efflux. Tyrosine produced a T_3 concentration gradient of 2.5 (one experiment).

Trans-acceleration of [^{125}I] T_3 influx was studied under initial-velocity conditions in the presence of various preloaded amino acids (10 mM), particularly those which are substrates of system T: tryptophan, phenylalanine and tyrosine. Initial velocities were calculated from the slopes obtained by linear regression (cell-associated radioactivity versus time), as described in the Experimental section. The ratio of the slopes relative to control was 1.3 ± 0.1 ($n = 4$) for L-tryptophan, 2.1 ± 0.1 ($n = 4$) for phenylalanine, 1.2 ($n = 2$; range 1.1–1.3) for tyrosine and 0.55 ± 0.06 ($n = 3$) for D-tryptophan. This last value, lower than 1, confirms that D-tryptophan *trans*-inhibits, rather than *trans*-accelerates, tryptophan uptake. Glycine, serine, glutamine, lysine, glutamic acid and taurine (10 mM) caused neither *trans*-acceleration nor *trans*-inhibition (ratio of the slopes = 1). These amino acids are respectively the main substrates of the amino acid-transport systems Gly, ASC, N, Ly^+ , Glu and β , present in human erythrocytes [33]. Thus binding to, or transport by, these carriers is not involved in *trans*-acceleration by aromatic amino acids. System L is also not involved, since 20 mM-leucine was present both intra- and extra-cellularly throughout the experiments.

Possible significance of T_3 /tryptophan interactions at the transport site

These kinetic data for tryptophan transport by the rat erythrocyte system T are fully compatible with a simple carrier model, as defined by Stein [30,31], and system T can drive the uptake of substrates against their concentration gradients. The rat erythrocyte system T exhibits unusual asymmetric exchange properties, resulting in *trans*-inhibition of tryptophan exit and *trans*-stimulation of tryptophan uptake, a behaviour also reported for system T in human erythrocytes [27]. Similar *trans* effects of tryptophan on the T_3 carrier (which was previously shown to exhibit pore-like kinetic properties [28]) are also observed. Since, in addition, the T_3 *trans*-inhibition of tryptophan transport is mediated by the T_3 carrier, there is no reason to assume any kinetic model more complicated than a common simple carrier for T_3 and tryptophan. T_3 *trans*-inhibition of T_3 and tryptophan transport would then result from either slow translocation (transconformation) of the T_3 -carrier complex (compared with that of the unloaded carrier) or slow dissociation of this complex at the membrane surfaces. Whether this kinetic model reflects the activity of a common carrier for both substrates or that of a set of interactive transport systems needs purification of the protein(s) implicated in the transport of T_3 and tryptophan and reconstitution of transport activities.

The system-T-mediated *trans*-acceleration of T_3 uptake by

intracellular aromatic amino acids induces active T_3 transport, resulting in the intracellular free T_3 concentration being higher than the extracellular concentration. The physiological significance of this mechanism, by which T_3 is pumped up its concentration gradient in rat erythrocytes, requires further study. It is not known if rat erythrocytes can accumulate sufficiently high intracellular levels of aromatic amino acids to maintain a sustained counterflow of T_3 . This would implicate other amino acid-exchanging systems (such as system L) and/or Na^+ -dependent amino acid-transport systems (such as system ASC). Both types of carrier are present in human erythrocytes [33], but rat erythrocytes are said to lack Na^+ -dependent carriers [34].

Countertransport, as an indirect mechanism for coupling T_3 uptake to the ionic gradients and, ultimately, to cellular energy, would be of great importance in target cells, since the hormone gradient across the plasma membrane might modulate T_3 binding to nuclear receptors. There are reports of system T-like transport activities in rat hepatocytes [35] and mouse blastocytes [36], but no indication as to whether they are linked to thyroid-hormone uptake. The existence of such a countertransport pathway, or a similar one, in target cells might explain some of the discrepancies in the roles of Na^+ and cellular ATP in thyroid-hormone uptake (see the Introduction). Such observations would depend on whether the cellular system used *in vitro* had kept all the components (particularly the gradient concentration of intracellular amino acids involved in countertransport) required to couple the T_3 flow to the Na^+ gradient and ATP hydrolysis. Erythrocytes provide a model system for studying the active mechanisms of T_3 uptake, and the implied hetero-exchange with intracellular amino acids.

Y.Z. is supported by a studentship from the Société de Secours des Amis des Sciences.

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Received 18 April 1991/11 June 1991; accepted 9 July 1991