

Red Blood Cell Triiodothyronine Uptake as Membrane Parameter of Depression

L. KALIŠOVÁ-STÁRKOVÁ, Z. FIŠAR, I. PACLT, Z. HANUŠ, J. VEVERA

Department of Psychiatry, First Faculty of Medicine, Charles University, Prague, Czech Republic

Received December 30, 2004

Accepted April 20, 2005

On-line May 24, 2005

Summary

We tested the hypothesis considering the role of hypothalamic-pituitary-thyroid axis (HPT), L-triiodothyronine (L-T3) uptake into erythrocytes, and the role of membrane lipids in the development and treatment of affective disorders. Changes in kinetic parameters (V_{\max} , maximal velocity and K_M , apparent Michaelis constant) of L-T3 uptake into red blood cells (RBCs) and changes in membrane fluidity in a group of 24 patients with major depression were measured before treatment and after 1 month of treatment with citalopram. Parameters V_{\max} and K_M , as well as membrane microviscosity, were significantly increased in depressed patients both before and after treatment in comparison with healthy subjects. We concluded that the function of the membrane transporter for L-T3 in RBC is changed in depression. This change is probably connected with alteration of membrane fluidity and/or transporter-lipid interactions. We did not find any normalization of the measured parameters after 1 month of treatment. The results show the importance of composition and physical properties of the lipid bilayer for transmembrane transport of L-T3 and support the hypothesis that the HPT axis is in depression.

Key words

Depression • Erythrocyte • Membrane fluidity • Triiodothyronine • Uptake

Introduction

The etiology of affective disorders has not yet been sufficiently clarified. One of the possible factors influencing the development of mood disorders might be due to the function of the hypothalamic-pituitary-thyroid (HPT) axis. Numerous reports focusing on patients who suffer from affective disorders, repeatedly confirmed aberrations of HPT axis function (Joffe and Levitt 1993, Sullivan *et al.* 1997). In thyrotropin-releasing factor (TRF) tests, approximately 30 % of clinically euthyroid depressive patients showed an altered response of the thyroid stimulating hormone (TSH) to TRF, transient

hyperthyroxinemia in the serum and a flattening of the TSH diurnal rhythm. In some cases, there were also lowered transthyretin levels in the cerebrospinal fluid, an increased level of antibodies against thyroid peroxidase and other responses (Bauer and Whybrow 2001, Kirkegaard and Faber 1998, Loosen *et al.* 1988, Pop *et al.* 1998, Styra *et al.* 1991). All the presented parameters seem to be “state markers” since after a patient has been cured of depression, the parameters tend to return to normal levels. However, the basic cause of these changes is not quite clear.

There is evidence that the mediator-system function is affected by the membrane transfer. The

changes in cell membranes may then reflect the changes on the system level. The present scientific knowledge suggests that erythrocytes, though they are not the target cells for thyroid hormones, can participate in their transport (Crispell and Coleman 1956, Yamauchi *et al.* 1989, Angel *et al.* 1990). Thyroxine (T4) enters the red blood cells (RBC) by free diffusion (Galton *et al.* 1996); L-T3 permeates through the erythrocyte membrane by facilitated diffusion, *via* the Na⁺ independent stereospecific saturable transport system (Holm and Jacquemin 1979, Docter *et al.* 1982, Dozin *et al.* 1985, Osty *et al.* 1990). Red blood cells seem to function as a reservoir pool of the hormone (Osty *et al.* 1988, 1990).

The function of membrane transport systems – velocity and efficiency of the L-T3 uptake into the erythrocytes – can be evaluated by two kinetic parameters: V_{\max} – maximal transport velocity into the cells, reflecting the existence of the saturable amount of membrane transporters; and K_M – the apparent Michaelis constant, defined as extracellular concentration of the transported substance, when the transport velocity into the cell equals to one half of V_{\max} . The K_M value is close to the value of dissociation binding constant of transporter–L-T3 complex, and K_M is also the affinity criterion between the transporter and substrate.

The activity of membrane transporters can be equally influenced by the properties of the lipid membrane component, mainly by the composition, structure and ordering of the lipid bilayer. The membrane microviscosity (reciprocal value of fluidity) is the parameter which characterizes rotational motility and average ordering of membrane molecules; the changes of the microviscosity can be qualitatively determined by means of the measurement of fluorescence anisotropy of an hydrophobic fluorescence probe. The parameter concerned serves to express relative changes of the membrane dynamic properties caused by alteration of membrane lipid composition or by physical or chemical factors (including effects of psychotropic drugs) (Hillier 1970, Samson *et al.* 1992, 1993).

An important modulator of microviscosity is the proportion of membrane phospholipids and cholesterol, the presence of unsaturated fatty acid side chains in membrane lipids, and the amount of oligosaccharides in membrane glycolipids and glycoproteins. 1,6-diphenyl-1,3,5-hexatriene (DPH) is the hydrophobic fluorescence probe most frequently used to study changes in the lipid bilayer microviscosity. Membrane microviscosity is a factor affecting both the lateral and transversal motion of

membrane proteins (receptors, ion channels, enzymes, transporters). Specific changes in their function can be caused by lipid-protein interactions. Special attention is paid to the role of cholesterol and acidic phospholipids (phosphatidylserine and phosphatidylinositol) in the activation of membrane components of signal transduction systems. As far as the L-T3 transporter in erythrocyte membranes is concerned, the dependence of their L-T3 binding parameters on phospholipid membrane composition has been described by Samson *et al.* (1996).

In our experiments we used RBCs isolated from peripheral human blood. Radiolabeled L-T3 uptake into erythrocytes was measured by the method which we had modified, and the kinetic parameters were measured before treatment, and after one month of treatment with citalopram (an antidepressant functioning primarily as a selective serotonin reuptake inhibitor). Subsequently, the results were compared with healthy controls. As the activity of the L-T3 transporter can be affected by the properties of the lipid part of the cell membrane, plasma membranes (ghosts) were isolated from erythrocytes, and using the fluorescence probe, changes in their lipid bilayer fluidity were identified. We tried to determine to what extent depression and administration of citalopram could affect the L-T3 uptake into erythrocytes and whether there exists a correlation between the membrane changes, L-T3 uptake parameters and clinical evaluation. These results evoked discussion of the hypothesis considering the role of HPT axis, L-T3 uptake into erythrocytes, and the role of the membrane lipids in the development and treatment of the affective disorders.

In our project we tried to verify the following assumptions: 1) L-triiodothyronine membrane transport of depressive patients differs from that of the healthy controls, and reflects changes on the systemic level, 2) the membrane transport system function is influenced by the change of membrane fluidity, and 3) the antidepressants return the parameters of depressive patients to normal levels.

Methods

Patients and the clinical evaluation of depression

Twenty-four subjects suffering from major depression participated in the project (diagnoses F 32.0-F 32.3.; F 33.1-F33.2 according to ICD 10; major depressive disorder single episodes or recurrent mild, moderate or severe – DSM IV). In this group (mean age

37±13 years, range: 19-60 years) there were 12 females (mean age 39±10 years) and 12 males (mean age 36±15 years). None of them, at the time when they joined the project, had been taking any antidepressants for at least five months. The majority of them were diagnosed as suffering from depression for the first time; 20 of them were 'drug naive'. The patients were clinically evaluated on the basis of a structured interview regarding valid diagnostic criteria with a psychiatrist at the Psychiatric Clinic in Prague, who made the diagnosis of major depression. Patients were then assessed using the Hamilton Depression Rating Scale (HAMD) (Hamilton 1960) and with the Beck Self-Assessment Depression Rating Scale (BECK) (Beck and Beamesderfer 1974). All the participants were comprehensively informed about the project, consented to the conditions and provided their written consent.

Between 8:00 and 10:00 h, after a clinical check-up, 7 ml of blood were withdrawn to examine free thyroxine (fT4) and TSH levels and 4 ml of non-coagulable blood were used for measuring the kinetic parameters of L-T3 uptake into erythrocytes and for subsequent measurements of membrane fluidity in the ghosts. The study included clinically euthyroid persons only; none of them had ever been treated with any HPT axis affecting medication. Standard values corresponded to 9.8-23.1 pmol/l for fT4 and 0.500-6.00 mIU/l for TSH. After a clinical check-up and initial sampling, all the depressive patients were treated with citalopram (Seropram, Citalec) in a dose of 20-60 mg/day. One month later (28-31 days), all the patients were clinically assessed with the HAMD and BECK and 4 ml of blood were taken for measurement of kinetic parameters of L-T3 uptake and for measurement of erythrocyte ghost membrane fluidity. In the course of the second evaluation and sampling, five patients were excluded from the project, as they no longer fulfilled the criteria of the study (for example they had changed an antidepressant, had discontinued the drug, were unable to attend the clinic at the planned time; etc.).

The control group consisted of subjects of corresponding age and the same nutritional status, who had not been treated either for psychiatric disorders or for thyroid gland dysfunction. There were 19 subjects in the control group, all of whom were screened during interviews with a psychiatrist for the presence of depressive symptoms. None of the controls fulfilled the criteria for major depression. A 4 ml sample of blood was taken from the controls for measurement of the L-T3

erythrocyte uptake kinetic parameters, and subsequent measurement of the membrane fluidity in erythrocyte ghosts.

Chemicals and solutions

800 nM [¹²⁵I] L-T3 solution with specific activity 16 kBq/ml was prepared by mixing of buffer T (125 mM NaCl, 20 mM KCl, 4 mM MgCl₂, 10 mM glucose, 4.05 mM Na₂HPO₄, 0.95 mM NaH₂PO₄; before use effervesced with 95 % O₂ and 5 % CO₂, pH 7) with unlabelled (cold) L-T3 (Sigma) solution and [¹²⁵I]L-T3 stock solution (specific activity of 114 MBq/μg, Amersham Pharmacia Biotech UK Ltd., England). Samples were filtered through glass microfiber filters (Whatman, type GF/C), which were impregnated in 0.1 % polyethyleneimine (Sigma) and filters were washed with buffer A (120 mM NaCl, 10 mM KCl, 30 mM Tris, 10 μM L-T3, pH 7.4). The hydrophobic membrane probe 1,6-diphenyl-1,3,5-hexatriene (DPH, Sigma) was used for the measurement of relative changes of plasma membrane fluidity.

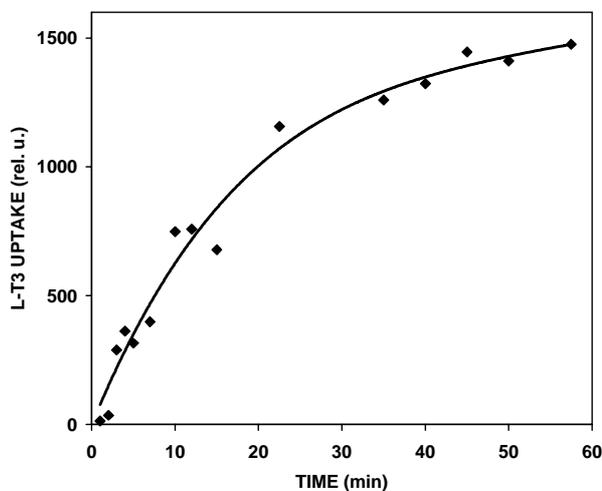


Fig. 1. Time course of L-T3 uptake into human erythrocytes. Intact erythrocytes (10^8 cells/ml) were incubated in buffer T in the presence of 40 nM [¹²⁵I]L-T3. One milliliter aliquots were rapidly filtered through GF/C glass fiber filters at selected time intervals. The filters were washed twice with ice-cold buffer A and measured by means of a scintillation counter.

Erythrocyte L-T3 uptake

Non-coagulable blood samples were mixed thoroughly and gently centrifuged in plastic test tubes; then the platelet-rich plasma was taken away together with the upper layer of the sediment and the RBCs pellet was washed three times with cold buffer T. The RBC

triiodothyronine uptake was measured using modified methods according to Osty *et al.* (1990) and Moreau *et al.* (1998, 2000). Briefly, RBCs in buffer T were preincubated at 37 °C for 12 min and the reaction was started by addition of various volumes of 800 nM [¹²⁵I] L-T3 solution. The final volume of the sample for the uptake measurement was 2 ml with concentration of the cells 10⁸ RBC/ml and the final L-T3 concentrations were in the range of 4 nM and 240 nM. The test tubes were filled with 95 % O₂ and 5 % CO₂ gas mixture, sealed and incubated at 37 °C for 15 min. It was verified that even after this time the increase of L-T3 uptake into the cells is approximately linear (Fig. 1). Free and non-specifically bound L-T3 was removed by rapid filtration through the GF/C filters and the filters were measured on a scintillation counter LS 6000IC (Beckman). Non-saturable L-T3 uptake (passive transport) was determined by measurement of the samples incubated at 2 °C.

Erythrocyte ghost fluidity

Plasma membranes from RBCs (ghosts) were isolated using the technique reported by Dodge *et al.* (1963). The ghosts were resuspended in 1.5 ml of buffer A, 10 µl were taken out for measuring phosphorus (Bartlett 1959, Wagner *et al.* 1962) and the sample was frozen. Membrane fluidity was measured using the method of fluorescence probes (Lakowicz 1999). The resultant DPH concentration was 2 µM, and the concentration of phospholipids was approximately 100 µM. Fluorescence anisotropy was measured on a spectrofluorometer FluoroMax-3 (Jobin Yvon, Horiba) after the 60 min incubation at 37 °C.

Data analysis

Erythrocyte L-T3 uptake is a passive saturable carrier-mediated transport process (facilitated diffusion) obeying simple Michaelis-Menten kinetics with an apparent Michaelis constant K_M and maximal velocity V_{max} . For calculation of the required kinetic parameters we used nonlinear regression analysis – the AccuFit Saturation Two-Site software (Beckman). Limiting permeability at low (physiological) L-T3 concentrations was calculated as V_{max}/K_M ratio. V_{max}/K_M is an efficiency criterion of the transport system.

Statistical Analysis

All values are presented as mean ± standard deviation (S.D.), or ± 95 % confidence interval (CONF95). The Wilcoxon matched pairs test was used to

compare the responses from the depressed patients before and after treatment with citalopram. The Mann-Whitney U test was used to compare the responses from the controls and from the depressed patients both before and after treatment with citalopram. Spearman R was used to quantify the relation between two quantitative parameters; pairwise deletion of missing data in correlation matrix was used. Normality of distribution was verified by the χ^2 test of the closeness of the fit and by the Kolmogorov-Smirnov one sample test. Application of t-tests and Pearson correlation coefficient results in very similar statistics as nonparametric methods mentioned above.

Results

Clinical assessment and parameters of erythrocyte L-T3 uptake were determined in 24 depressive patients before the beginning of treatment and in 19 of them after one month of pharmacotherapy with citalopram taken at a daily dose 20-60 mg. Following one month of treatment with citalopram, the depressive patients showed a significant decrease in BECK ($p=0.00013$) and HAMD ($p = 0.00013$) score (Table 1). The depressive patients before treatment showed significantly enhanced kinetic parameters of RBC L-T3 uptake in comparison with the controls (Table 2) both for K_M ($p=0.026$) and V_{max} ($p=0.0077$). After one-month treatment with citalopram the values of uptake parameters remained significantly increased compared to the control values ($p=0.0090$ for K_M ; $p=0.015$ for V_{max}). We detected no statistically significant change of the measured parameters after one-month treatment in comparison with the values before the beginning of citalopram administration both for K_M ($p=0.63$) and V_{max} ($p=0.65$). When the data from the females and males were processed separately, the results remained qualitatively unchanged.

From the values K_M and V_{max} the limiting permeability was calculated (V_{max}/K_M) at low (physiological) L-T3 concentrations (Table 2). Compared to the controls, there were no significant changes of this parameter either in depression before the treatment ($p = 0.54$), or after the treatment with citalopram ($p = 0.34$). The relatively greatest, although statistically not significant, change of V_{max}/K_M appeared in comparison of values before and after the treatment ($p = 0.21$).

Table 1. Age and clinical assessment of depression in depressed patients before and after one month treatment with citalopram.

	n	Age (years)	BECK score	HAMD score
<i>Depressed patients before treatment</i>	24	37.3 ± 12.6	16.1 ± 7.5	21.3 ± 4.0
<i>Depressed patients treated one month with citalopram</i>	19	38.5 ± 12.3	7.8 ± 4.8***	6.8 ± 3.1***
<i>Controls</i>	19	35.6 ± 10.5	–	–

Values are given as mean ± S.D. Values marked by *** are significantly different ($p < 0.001$) from patients before treatment (Wilcoxon test).

Table 2. Kinetic parameters of RBC L-T3 uptake and fluorescence anisotropy of DPH in ghosts of depressed patients before and after one month of treatment with citalopram

		K_M	V_{max}	V_{max}/K_M	r_{DPH}
<i>Depressed patients before treatment</i>	mean	166.5*	5.77**	0.0356	0.2323*
	S.D.	68.2	2.49	0.0101	0.0065
	CONF95	27.3	0.99	0.0040	0.0026
	n	24	24	24	24
<i>Depressed patients treated for one month with citalopram</i>	mean	176.0**	5.48*	0.0315	0.2316
	S.D.	68.4	2.44	0.0095	0.0083
	CONF95	30.8	1.10	0.0043	0.0039
	n	19	19	19	18
<i>Controls</i>	mean	116.1	3.67	0.0345	0.2279
	S.D.	62.1	1.77	0.0105	0.0066
	CONF95	27.9	0.80	0.0047	0.0029
	n	19	19	19	20

V_{max} – maximal velocity of L-T3 uptake to RBC ($\text{pmol}/\text{min} \cdot 10^8$ cells); K_M – apparent Michaelis constant reflecting extracellular concentration of L-T3, when the transport velocity into cells equals to one half of V_{max} (nM); V_{max}/K_M – limiting permeability at low L-T3 concentrations (min^{-1}); r_{DPH} – fluorescence anisotropy of 1,6-diphenyl-1,3,5-hexatriene (DPH), S.D. – standard deviation, n – number of values, CONF95 – 95 % confidence interval for the mean. Significant differences from controls: * $p < 0.05$, ** $p < 0.01$ (Mann-Whitney U test). There were no significant differences in uptake parameters and fluorescence anisotropy before and after treatment.

Fluorescence anisotropy of DPH (r_{DPH}) in ghosts of depressive patients before the treatment was found statistically significantly increased compared to the controls ($p = 0.030$; Table 2). After one-month of treatment with citalopram only a slight decrease in this parameter was observed ($p = 0.11$).

Mutual relations between the measured parameters were tested by means of correlation coefficients matrix (Table 3). A significant correlation between the clinical evaluation before treatment and after one-month citalopram administration was present; the correlation was determined using the score of HAMD or BECK. Significant correlations were also observed between K_M and V_{max} values, both before and after the treatment; the same relation between K_M a V_{max} is also

valid for the controls. No significant correlation was found between fluorescence anisotropy of DPH in ghosts and other measured variables.

Discussion

The data presented here confirm the significance of RBCs in blood transport and homeostasis of L-T3. Our data show that kinetic parameters of L-T3 uptake into erythrocytes were increased in depressive patients before treatment when compared with the controls group. In the course of pharmacotherapy, these parameters did not change significantly (Table 2). All the patients were euthyroid and were responding well to the treatment (Table 1). Later, we detected enhanced fluorescence

Table 3. Relations between clinical evaluation, parameters of L-T3 uptake and fluorescence anisotropy of DPH of depressed patients before and after treatment with citalopram determined as correlation coefficients (Spearman R).

	Before treatment					After one month treatment with citalopram					
	BECK 1	$K_M 1$	$V_{max} 1$	$V_{max} 1/K_M 1$	$\Gamma_{DPH} 1$	HAMD 2	BECK 2	$K_M 2$	$V_{max} 2$	$V_{max} 2/K_M 2$	$\Gamma_{DPH} 2$
HAMD 1	0.652**	-0.268	-0.080	0.226	0.372	0.666**	0.428	0.053	-0.258	-0.436	-0.063
BECK 1	1.000	-0.464*	-0.466*	-0.086	0.212	0.339	0.583**	-0.089	-0.075	-0.301	-0.065
$K_M 1$		1.000	0.850**	-0.178	0.097	-0.176	-0.339	0.223	0.119	0.114	0.164
$V_{max} 1$			1.000	0.292	-0.058	0.034	-0.337	0.174	0.117	0.225	0.026
$V_{max} 1/K_M 1$				1.000	-0.293	0.202	-0.172	-0.088	-0.040	0.153	-0.172
$\Gamma_{DPH} 1$					1.000	0.166	0.344	-0.172	-0.360	-0.284	0.061
HAMD 2						1.000	0.543*	-0.041	-0.229	-0.405	0.031
BECK 2							1.000	-0.001	-0.008	-0.108	0.143
$K_M 2$								1.000	0.783**	-0.016	0.094
$V_{max} 2$									1.000	0.591**	-0.172
$V_{max} 2/K_M 2$										1.000	-0.360

HAMD – Hamilton Depressive Rating Scale score, BECK – Beck Self-Assessment Depression Rating Scale score, K_M – apparent Michaelis constant for L-T3 uptake to RBC (nM), V_{max} – maximal velocity of L-T3 uptake to RBC (pmol/min $\cdot 10^8$ cells), V_{max}/K_M – limiting permeability at low L-T3 concentrations (min⁻¹), Γ_{DPH} – fluorescence anisotropy of DPH in erythrocyte ghosts. Variables measured before treatment are marked with No. 1, after one month treatment with citalopram are marked with No. 2. Asterisks indicate significant correlations: * p<0.05 or ** p<0.01

anisotropy of the DPH probe in erythrocyte membranes of depressive patients both before and after the one-month treatment with citalopram (Table 2). As fluorescence anisotropy of DPH reflects the degree of the probe movement restriction in an anisotropic membrane environment, and the same applies to the degree of movement restriction of membrane molecules. We can thus assume that the change of kinetic parameters of L-T3 uptake into erythrocytes during depression is to a certain extent affected by changes of the interaction lipid-L-T3 transporter.

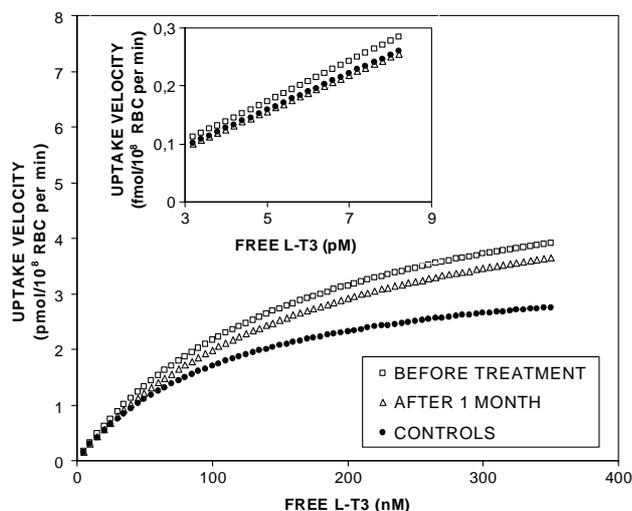


Fig. 2. Uptake L-T3 into erythrocytes at low and high concentrations of free L-T3. Velocities of L-T3 transport are calculated from K_M and V_{max} values stated above in Table 2. \square – Depressive patients before treatment ($K_M = 166.5$ nM, $V_{max} = 5.77$ pmol/min $\cdot 10^8$ cells), \triangle – depressive patients after one month of citalopram administration ($K_M = 176.0$ nM, $V_{max} = 5.48$ pmol/min $\cdot 10^8$ cells), \bullet – controls ($K_M = 116.1$ nM, $V_{max} = 3.67$ pmol/min $\cdot 10^8$ cells).

We did not find significantly different values of K_M , V_{max} or r_{DPH} in patients diagnosed as depressive for the first time ($n=20$), and in patients who had already been treated (but they were more than 5 months before the beginning of the project without medication $n=4$).

The interpretation of altered kinetic parameters of L-T3 uptake is not completely unequivocal. Higher V_{max} can reflect higher density of transporters in the membrane, or it can only mean their higher activity while the density is unchanged. The enhanced activity might be caused e.g. by specific interaction with certain membrane molecules and/or by altered dynamic properties of the lipid bilayer, in which transporters are embedded. The increased K_M value corresponds qualitatively to the

decrease in L-T3 transporter affinity, i.e. it corresponds to the decrease in its capacity to bind free L-T3, in particular at low concentrations. The decrease in affinity of the binding site for L-T3 can be considered either as a result of the conformation change of the binding site caused by allosteric interaction with other molecules, or as a result of the deeper embedding of the whole carrier protein into the lipid bilayer, and consequently altered accessibility of the binding site.

While interpreting the changes of kinetic parameters of L-T3 uptake, it is necessary to take into account the physiological concentrations of transported ligand. As for the free L-T3, the standard values move within the interval of 3.28-8.20 pmol/l. Considering that the detected K_M constants are within the range of tens to hundreds of nmol/l, the L-T3 transporter is in the entirely non-saturated state. At low (physiological) concentrations of free L-T3, the effectiveness of its uptake depends on the affinity of L-T3 transporter for its substrate. V_{max} enhancement in depressive patients can then be easily compensated by an increase of K_M (lowering of affinity). This can cause a situation when the significant changes of kinetic parameters of L-T3 uptake cause a higher rate of transport into erythrocytes at nanomolar concentrations of free L-T3, while at its picomolar concentrations the velocity of transport is virtually unchanged (Fig. 2). The change of kinetic parameters of L-T3 uptake into RBC in depression is, according to our findings, compensated in this way and it is possible to ask to what extent the V_{max} and K_M change is the starting factor, or just an associated symptom of depression.

Our data enable us to discuss the role of L-T3 uptake into RBC in the development and treatment of depressive disorders, both for the changes of kinetic parameters of uptake, and changes of dynamic properties of the lipid bilayer, in which the transport protein for L-T3 is embedded. Our study was stimulated by the results of Moreau *et al.* (1998, 2000), although we did not confirm them entirely. The differences in absolute values of measured kinetic parameters K_M and V_{max} in our and Moreau's study can easily be explained: we used a different method of separation of extracellular and intracellular L-T3. Compared to their centrifugation technique, our technique of rapid filtration has a certain advantage because we could wash away the nonspecifically surface-bound L-T3 more rapidly. However, a different method should not influence the marked qualitative decrease in measured parameters detected by Moreau *et al.* (2000) after a four-week treatment with

fluvoxamine. Our results confirm, in accordance with Moreau's study, a significant increase in kinetic parameters of L-T3 uptake into RBC in patients suffering from depression before the beginning of the treatment (drug-free state); all the patients engaged in our study were responders. However, after one-month treatment with citalopram we did not find any significant changes in the measured parameters, i.e. they were not normalized, although the score of HAMD and BECK in all the cases dropped remarkably. By comparing both sets in terms of the clinical parameters, we found that Moreau's study dealt with a slightly different group of patients – the level of depression may have been equally chosen (HAMD \pm S.D. was 33.7 ± 9.6) (but Moreau used a 26 degree Hamilton scale, we used a 21 degree scale), only 8 patients were drug-naïve, the washout period prior to the treatment was only 7 days, and after one month 9 patients were qualified as non-responders.

Our data do not sufficiently answer all the questions, although they enable us to sum up that the kinetics of L-T3 uptake into RBC can be seen as a process, which becomes altered in depression but does not correlate directly with the depressive symptoms. Most probably the point is the change of activity of membrane L-T3 transporter that is caused by altered properties of the lipid bilayer, in which the carrier protein is embedded. This conclusion is supported by the changes of fluorescence anisotropy of DPH in plasma membranes from erythrocytes, which shows that the depressive disorder is accompanied by a lowered fluidity of the membranes concerned. Cell membrane dynamics affect various membrane enzymes, receptors, ion channels and transporters; one of the manifestations of its change could be the increase in kinetic parameters of L-T3 uptake. This conclusion is supported by the results of our another project (in progress), in which we measured [125 I]L-T3 uptake into erythrocytes in patients with hypercholesterolemia. The increased plasma levels of cholesterol significantly correlated with the increased anisotropy of fluorescence of DPH in erythrocyte membranes compared to the controls (mean \pm S.D.; r_{DPH} 0.2376 ± 0.0071 vs. 0.2279 ± 0.0066 , $p < 0.001$), and the kinetic parameters of [125 I]L-T3 uptake into erythrocytes

were also significantly increased compared to the controls (K_M 224.1 ± 127.1 vs. 116.1 ± 62.1 nM, $p = 0.00057$; V_{max} 5.91 ± 2.97 vs. 3.67 ± 1.77 pmol/min $\cdot 10^8$ cells, $p = 0.0072$). The velocity of L-T3 uptake into erythrocytes was generally decreased at increased membrane microviscosity, which was determined by limiting permeability reflecting kinetics of uptake at physiological L-T3 concentrations (V_{max}/K_M 0.0278 ± 0.0062 vs. 0.0345 ± 0.0105 min $^{-1}$, $p = 0.024$). It has not yet been fully elucidated, whether the characteristics of L-T3 transporter are influenced by specific lipid-protein interactions (e.g. cholesterol transporter, phosphatidyl-serine transporter or others), rather than by the overall dynamic state of the lipid bilayer, i.e. by arrangement and rotational and translational motility of the membrane molecules.

Clinically, the work supports the hypothesis according to which we can evaluate the triiodothyronine uptake into erythrocytes as a peripheral membrane marker of a depressive disorder. The hypothesis of altered function of the hypothalamic-pituitary-thyroid axis in the development and maintenance of depression points towards medication of thyroid hormones for effective therapy for depression.

Our results support the hypothesis that even clinically euthyroid patients with a depression show a change of L-T3 uptake into erythrocytes. It can be speculated that L-T3 uptake into erythrocytes can be used as a subclinical marker reflecting the dysfunction of the hypothalamic-pituitary-thyroid axis in pathophysiology of affective disorders. Nevertheless, the kinetic parameters of L-T3 uptake into erythrocytes do not correlate with depressive symptoms and are affected by properties of the lipid bilayer. We have tried to combine the hypothesis concerning the erythrocyte L-T3 uptake alteration with the membrane hypothesis of affective disorders, according to which the vulnerability to depression can originate from changes in the composition and biophysical properties of the lipid component of cell membranes.

Acknowledgements

This work was supported by GA UK No. 27/2000/C and MSM 111100001 grants.

References

- ANGEL RC, BOOTTA JA, MORERO RD, FARIAS RN: Solubilization and purification of membrane erythrocytes associated 3,3',5 triiodo-L-thyronine binding protein from rat. *Biochem J* **270**: 577-582, 1990.
- BARTLETT GR: Phosphorus assay in column chromatography. *J Biol Chem* **234**: 466-468, 1959.

- BAUER M, WHYBROW PC: Thyroid hormone, neural tissue and mood modulation. *World J Biol Psychiatry* **2**: 59-69, 2001.
- BECK AT, BEAMESDERFER A: Assessment of depression inventory. In: *Psychopharmacology*, P PICHOT (ed), Basel, 1974, pp 151-169.
- CRISPELL KR, COLEMAN J: A study of the relative binding capacity of plasma proteins, intact human red cells, and human red cell stroma for radioactive I-131 labeled L-thyroxine. *J Clin Invest* **35**: 475-480, 1956.
- DOCTER R, KRENNING EP, BOS G, FEKKES DF, HENNEMANN G: Evidence that the uptake of triiodo-L-thyronine is carrier-mediated, but not energy-dependent. *Biochem J* **208**: 27-34, 1982.
- DODGE JT, MITCHELL C, HANAHAN DJ: The preparation and chemical characteristics of hemoglobin-free ghosts of human erythrocytes. *Arch Biochem Biophys* **100**: 119-130, 1963.
- DOZIN B, CAHNMANN HJ, NIKODEM VM: 1985. Comparative characterization of thyroid hormone receptors and binding proteins in rat liver nucleus, plasma membrane and cytosol by photoaffinity labeling with L-thyroxine. *Biochemistry* **24**: 5203-5208, 1985.
- GALTON VA, ST GERMAIN DL, WHITTEMORE S: Cellular uptake of cells. 3,5,3' triiodothyronine and thyroxine by red blood and thymus cells. *Endocrinology* **118**: 1918-1923, 1996.
- HAMILTON MA: A rating scale for depression. *J Neurol Neurosurg Psychiatr* **23**: 56-62, 1960.
- HILLIER AP: The binding of thyroid hormone to phospholipid membranes. *J Physiol Lond* **211**: 585-597, 1970.
- HOLM AC, JACQUEMIN C: Membrane transport of L-triiodothyronine by human red cell ghosts. *Biochem Biophys Res Commun* **89**: 1006-1017, 1979.
- JOFFE RT, LEVITT AJ: *The Thyroid Axis and Psychiatric Illness*. American Psychiatry Press, Washington, DC, 1993, p 339.
- KIRKEGAARD C, FABER J: The role of thyroid hormone in depression – review. *J Endocrinol* **138**: 1-9, 1998.
- LAKOWICZ JR: *Principles of Fluorescence Spectroscopy*, Kluwer Academic/Plenum Publishers, New York, 1999.
- LOOSEN PT, ARTHUR J, PRANGE JR: Serum thyrotropine response to thyrotropine releasing hormone in psychiatric patients: a review. *Am J Psychiatry* **139**: 405-410, 1988.
- MOREAU X, AZORIN JM, MAUREL M, JEANNINGROS R: Increase in red blood cell triiodothyronine uptake in untreated unipolar major depressed patients compared to healthy volunteers. *Prog Neuropsychopharmacol Biol Psychiatry* **22**: 293-310, 1998.
- MOREAU X, AZORIN JM, LEJEUNE PJ, JEANNINGROS R.: Red blood cell triiodothyronine uptake in unipolar major depression: effect of a chronic antidepressant treatment. *Prog Neuropsychopharmacol Biol Psychiatry* **24**: 23-35, 2000.
- OSTY J, JEGO L, FRANCON J, BLONDEAU JP: Characterization of triiodothyronine transport and accumulation in rat erythrocytes. *Endocrinology* **123**: 2303-2311, 1988.
- OSTY J, VALENSI P, SAMSON M, FRANCON J, BLONDEAU JP: Transport of thyroid hormones by human erythrocytes: kinetic characterization in adults and newborns. *J Clin Endocrinol Metab* **71**: 1589-1595, 1990.
- POP JK, LUC HM, LEUSINK G, VAN SON MJ, KOTTNERUS AJ, WARD M: Are autoimmune thyroid dysfunction and depression related? *J Clin Endocrinol Metab* **83**: 3194-3197, 1998.
- SAMSON M, OSTY J, FRANCON J, BLONDEAU JP: Triiodothyronine binding sites in the rat erythrocyte membrane: involvement in triiodothyronine transport and relation to the tryptophan transport system T. *Biochim Biophys Acta* **1108**: 91-98, 1992.
- SAMSON M, OSTY J, BLONDEAU JP: Identification by photoaffinity labeling of a membrane thyroid hormone binding protein associated with the triiodothyronine transport system in rat erythrocytes. *Endocrinology* **132**: 2470-2476, 1993.
- SAMSON M, OSTY J, THIBOUT H, BLONDEAU JP: Solubilization, reconstitution and molecular properties of the triiodothyronine transport protein from rat erythrocyte membranes. *Eur J Endocrinol* **134**: 660-668, 1996.
- STYRA R, JOFFE R, SINGER W: Hyperthyroxinemia in major affective disorders. *Acta Psychiatr Scand* **81**: 61-63, 1991.
- SULLIVAN PF, WILSON DA, MULDER RT, JOYCE PR: The hypothalamic-pituitary-thyroid axis in major depression. *Acta Psychiatr Scand* **93**: 370-378, 1997.

WAGNER H, LISSAU A, HOLZI J, HORAMMER L: The incorporation of ^{32}P into inositolphosphatides of the rat brain. *J Lipid Res* **3**: 177-180, 1962.

YAMAUCHI K, HORIUCHI R, TAKIKAWA H: Uptake of 3,5,3'-triiodothyronine in human erythrocytes. *J Endocrinol* **121**: 585-591, 1989.

Reprint requests

L. Kališová-Stárková, Department of Psychiatry, First Faculty of Medicine, Charles University, Ke Karlovu 11, 128 01, Prague 2, Czech Republic. Fax: +420-224923077. E-mail: lucie_starkova@yahoo.com