

Imipramine Distribution among Red Blood Cells, Plasma and Brain Tissue

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Abstract. The relations between the concentrations of a drug in the blood (plasma, haemoglobin, red blood cells (RBCs), RBC membranes) and in the brain tissue (homogenate, membranes, cytosol) were investigated during chronic administration of imipramine. Radioimmunoassay was employed to measure the antidepressant concentrations. The concentrations were measured and analysed in 40 rats receiving various doses of imipramine. The concentration of total imipramine (imipramine + desipramine) in erythrocyte membranes (ghosts) amounted to $79.4 \pm 4.6\%$ (mean \pm S.E.M., $N = 40$) of those measured in intact RBCs. Marked accumulation of the drug in the brain tissue, especially in brain cell membranes, was confirmed. The concentrations in brain tissue homogenate was found to be 14.8 times higher than that in RBCs. Values in brain membranes were 10.9 times higher than that in blood element membranes. There is a significant association between the concentrations measured in brain homogenate, the blood plasma and RBC membranes. Blood concentrations can be used to estimate imipramine concentrations in the brain.

Key words: Imipramine — Brain — Blood — Membrane

Introduction

Tricyclic antidepressants such as imipramine (IMI) and desipramine (DMI) are cationic-amphiphilic drugs found after administration in the plasma (free or protein-bound), in cell membranes (mainly in lipid bilayer) and in cytosol. Major active metabolites of IMI are DMI, 2-hydroxy-IMI, and 2-hydroxy-DMI. Simultaneously, the rate of hydroxylation is the rate limiting step of the drug elimination. The role of these hydroxy-derivatives in drug therapy was discussed by Glassman (1994).

The plasma levels of IMI and its metabolites are known to exhibit major interindividual differences; these levels cannot be fully correlated with the admin-

istered dose of the drug. However, impressive evidence is available for an association between the plasma levels of IMI and the therapeutic response (Åsberg and Sjöqvist 1978; Perel et al. 1978; Risch et al. 1979; Glassman 1994). Statistically significant relationships between the therapeutic response and the concentration of total imipramine (IMI+DMI) in the plasma were observed between 175 and 350 ng/ml; with DMI, such an association has been demonstrated at DMI concentrations > 116 ng/ml (Perry et al. 1994). It was hypothesized that depression subtypes (unipolar and bipolar) affect the steady-state plasma levels of tricyclic antidepressants (Musa 1979, 1981). Intracellular storage of these drugs presumably induces changes in the phospholipid composition of cell membranes (Moor et al. 1988; Toplak et al. 1990), and it may be related to toxicity (Ruben et al. 1993).

It has been suggested that the therapeutic effects of antidepressants are accomplished through the interaction between the drug and the plasma membranes of target cells. The antidepressant action is associated with the membranes in terms of receptors and neurotransmitter reuptake (Marcusson and Ross 1990; Caldecott-Hazard et al. 1991; Graham and Langer 1992), ion transmission, the activity of several enzymes, and transmembrane signal transduction (Ackenheil 1990; Wachtel 1990; Manji 1992; Yamamoto et al. 1992; Hudson et al. 1993). Also, the physical and chemical properties of antidepressants make it possible for the antidepressants to bind into lipid bilayer. The concentrations in the lipid parts of biological membranes may exceed, by several orders, the concentrations in body fluids. It is possible that the therapeutic action of antidepressants is effected via their interactions with membrane phospholipids (Bauer et al. 1990; Fišar et al. 1991; Mason et al. 1991; Seydel et al. 1994).

In the blood amphiphilic drugs accumulate in red blood cell (RBC) membranes; this explains the efforts to study the binding of antidepressants to RBCs both *in vitro* (Bickel 1975; Javaid et al. 1985) and in depressed patients (Linnoila et al. 1978; Matuzas et al. 1983; Aymard et al. 1993). As the actual effects of psychotropic drugs take place in the brain, the concentrations and the pharmacokinetics of antidepressants in specific areas of the rat brain have also been investigated. Differences were found both in drug distribution between various brain regions and between acute and chronic IMI treatment regimens (Nagy 1977; Daniel et al. 1981, 1982; Friedman and Cooper 1983; Masada et al. 1986; Miyake et al. 1990). Specific brain regions responsible for the therapeutic effect of antidepressants are not known. Repeated administration of IMI to rats resulted in an increase of its plasma levels. A maximum was reached on day 3 and the value did not change further (Sikora et al. 1990).

The present study is aimed at determination of the association between blood and brain concentrations of antidepressant drugs. The partition of the drugs between water phase (plasma, cytosol) and membrane was considered. Concentration of IMI plus its active metabolite, DMI was measured not only in the plasma, RBCs

and brain homogenate but also in RBC membranes, haemoglobin, brain plasma membranes and brain cytosol.

Attention was paid to quantification of the accumulation of the drug in membranes. Membrane concentrations of the drug were related to the membrane phospholipid contents rather than to membrane proteins. This made possible a correct comparison of concentrations in RBC membranes and in brain membranes.

The concentrations of IMI+DMI measured in different compartments of the brain tissue and the blood were statistically evaluated. A formula was suggested to calculate IMI+DMI concentrations in the rat brain from those in the blood. This analysis may help clarify the fact that sometimes the plasma concentrations of the drug do not correlate well with the therapeutic effects of the antidepressant.

Abbreviations: IMI – imipramine, 5-(3-dimethylaminopropyl)-10,11-dihydro-5H-dibenz[b,f]azepine; DMI – desipramine, 10,11-dihydro-5-(3-methylaminopropyl)-5H-dibenz[b,f]azepine; RBC – red blood cell; R_PLA – plasma concentration [ng/ml]; R_HEM – haemoglobin concentration [ng/ml]; R_RBC – concentration in RBC mass (pellet) [ng/ml]; R_GHO – concentration in RBC ghosts [ng/ml] (related to ml of RBC mass); R_MEM – concentration in RBC membranes [ng/ml] (related to ml of phospholipid membranes supposing a membrane density of 1 g/cm³); B_HOM – concentration in brain homogenate [ng/ml]; B_MEX – concentration in brain membranes [ng/ml] (related to ml of membrane suspension); B_MEM – concentration in brain membranes [ng/ml] (related to ml of phospholipid membranes); B_CYT – concentrations in brain cytosol [ng/ml].

Materials and Methods

Various doses (4; 8; 12; 18; 26; 32; 34; 38 mg/kg.day) of IMI hydrochloride (SIGMA) were administered to forty laboratory Wistar rats (SPS breed) orally as an aqueous solution. Chronic treatment consisted of administration for seven days. The animals were anaesthetized with ether, and blood from the heart was collected into tubes containing EDTA (0.1%). After opening the skull, the brain was removed. Using radioimmunoassay (RIA) IMI+DMI concentrations in the blood and in the brain tissue were measured. The samples were processed and measured in pairs. The results were statistically analysed (correlation, partial correlation, linear regression and multiple linear regression analysis).

Blood processing

Whole blood (1.5 ml) was centrifuged (14,000 × *g*, 10 min, 20°C); a sample (10 μl) was collected from the plasma to measure the drug concentration (R_PLA). The remaining plasma was removed, the pellet was resuspended in 1.5 ml of saline, immediately centrifuged (14,000 × *g*, 10 min, 20°C); the supernatant was removed

and a sample (100 μl) from the pellet was collected for measurement (R_RBC). An aliquot of 150 μl of the pellet was hemolyzed by adding 1350 μl of ice water; the hemolyzate was centrifuged ($14,000 \times g$, 10 min, 20°C) and a sample (100 μl) to be measured was collected from the supernatant containing 10 times diluted haemoglobin (R_HEM). The pellet (erythrocyte ghosts) was resuspended in 150 μl of water thus diluting ghosts 2.08 ± 0.06 times (mean \pm S.E.M., $N = 6$, determined by gravimetry); a sample (100 μl) was collected for measurement (R_GHO). Another sample (10 μl) was collected for the determination of phosphorus concentration used to calculate the drug concentration in the phospholipid bilayer (R_MEM).

Brain tissue processing

The whole brain was weighed and homogenized in four volumes of saline (supposing a tissue density of 1 g/cm^3) with a glass homogenizer and a Teflon piston, and a sample (50 μl) to be measured was collected (B_HOM). The homogenate was centrifuged ($90 \times g$, 10 min, 4°C) and the supernatant was centrifuged again ($20,000 \times g$, 20 min, 4°C). The supernatant was removed and the pellet was rehomogenized in five volumes of ice water (ULTRA-TURAX, using an 8N probe for 3–5 s at high revolutions). The homogenate was centrifuged ($48,000 \times g$, 20 min, 4°C) and a sample (100 μl) of six times diluted cytosol to be measured was collected from the supernatant (B_CYT). The pellet was weighed and diluted with 10 volumes of saline; a sample (100 μl) to be measured was collected (B_MEX). Another sample was collected (50 μl) for phosphorus concentration measurement; the value was used to calculate the drug concentration in the phospholipid bilayer (B_MEM).

All samples were obtained and measured in pairs.

Radioimmunoassay

Radioimmunological determination of tricyclic antidepressants (Krulik et al. 1991a) was used in our study. Samples for RIA measurement were used either without any further processing (plasma, haemoglobin, cytosol) or the antidepressant had first been extracted (RBCs, RBC membranes, brain homogenate, brain membranes). Samples were stored at -50°C .

^3H -imipramine (^3H -IMI, 2.6 TBq/mmol) was used as radioligand. The concentrations of "total imipramine" (IMI+DMI) were measured as the cross reactivity values of the antiserum used; they were 94% for DMI, but near zero both for 2-hydroxyimipramine and 2-hydroxydesmethyylimipramine. Standard error of the method was $5.3 \pm 0.5\%$ ($N = 17$). The detection limit of the method employed was 0.5 ng/ml.

Drug extraction

Samples were alkalinized by adding 20 to 40 μl of 0.1 mol/l NaOH. The suspension was agitated for 5 min in polyethylene micro test tubes with 1 ml of *n*-heptane containing 0.3% isoamylalcohol. After centrifugation at $14,000 \times g$ for 5 min, 500 μl of the heptane phase was agitated for 5 min with 200 μl of 0.01 mol/l HCl. The mixture was centrifuged at $14,000 \times g$ for 5 min. The heptane phase was removed and 50 μl of the aqueous phase was collected. Extraction was controlled using tritium-labelled imipramine incubated with some samples (37°C, 20 hours) as a tracer. The extraction yield was $86 \pm 2\%$ (mean \pm S.E.M., $N = 26$, at blood IMI concentrations ranging between 10 and 1000 ng/ml); consequently the results were corrected by dividing them by 0.86.

Sample measurement

Aliquot parts of examined samples, control plasma, buffer (0.1 mol/l phosphate buffer with 0.01% NaN_3 and with 0.1% gelatine, pH 7.4), ^3H -IMI (0.3 pmol/sample) and diluted antiserum (1:100) were mixed, incubated and processed as described by Krulík et al. (1991a). Measurements were performed using an LS 6000IC scintillation counter (Beckman Instruments, Inc.).

Analysis of phosphorus

The phospholipid contents of erythrocyte or brain membrane suspensions were determined by a method, in which the phosphate content of the sample was first measured (Bartlett 1959). With this method, phospholipid phosphorus is acid-hydrolysed to inorganic phosphate; this is converted to phosphomolybdenic acid and reduced to blue colours, and measured photometrically.

Data processing

Drug concentrations were calculated with the help of a program (ImmunoFit EIA/RIA, Beckman) using a 4-parameter logistic model for standard curve. Results were statistically analysed using STATGRAPHICS software (STSC, Inc.). Drug concentrations in erythrocyte ghosts or in brain membrane suspensions were corrected for unbound drug by subtraction of concentrations found in diluted haemoglobin or brain cytosol.

Drug concentrations were expressed either in ng per 1 ml of sample (plasma, RBCs, haemoglobin, brain cytosol, brain homogenate) or in ng per 1 ml of the phospholipid membrane supposing a membrane density of 1 g/cm^3 (RBC membranes, brain membranes).

Results

The concentrations of drugs were measured and analysed in 40 rats receiving IMI at average doses ranging from 4 to 38 mg/kg.day. The broad range of doses of the drug was indispensable for statistical analysis of associations between the drug concentrations in various components of the blood and the brain tissue.

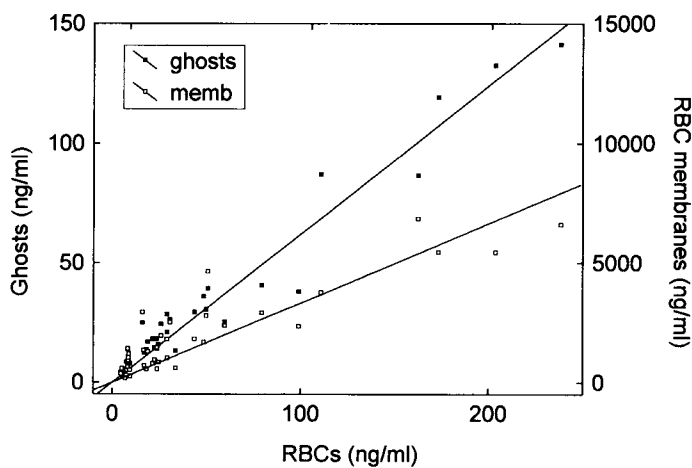


Figure 1. IMI+DMI concentrations in erythrocyte ghosts or RBC membranes plotted vs concentrations in RBCs.

The used doses of IMI resulted, after 7 days of administration, in plasma IMI+DMI concentrations ranging between 3 and 338 ng/ml. IMI and DMI accumulated in cellular membranes, mainly in their lipid parts. The concentrations of both drugs in erythrocyte ghosts (R_GHO), i.e. after haemoglobin removal, were $79.4 \pm 4.6\%$ (mean \pm S.E.M., $N = 40$) of the concentrations found in intact RBCs (R_RBC; Fig. 1). Actual IMI+DMI concentrations in lipid bilayer (R_MEM; in ng per ml of the phospholipid membrane) can be calculated from R_GHO (in ng per ml of ghosts suspension):

$$R_MEM = R_GHO / PL \quad (1)$$

where PL is the phospholipid concentration (in g/ml) in membrane suspension. PL was measured for each sample. A similar procedure was used to calculate IMI+DMI concentrations in brain membranes (B_MEM; in ng per ml of the phospholipid membrane) from the concentrations in brain membrane suspensions (B_MEX; in ng per ml of membrane suspension). This calculation makes it possible to compare the

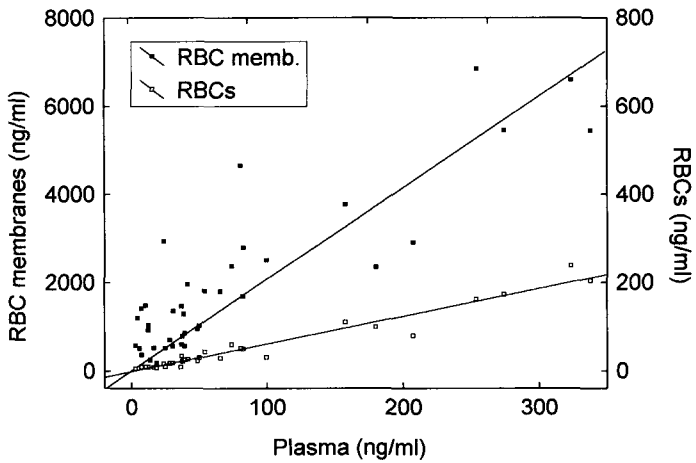


Figure 2. IMI+DMI concentrations in RBC membranes or intact RBCs plotted vs blood plasma concentrations.

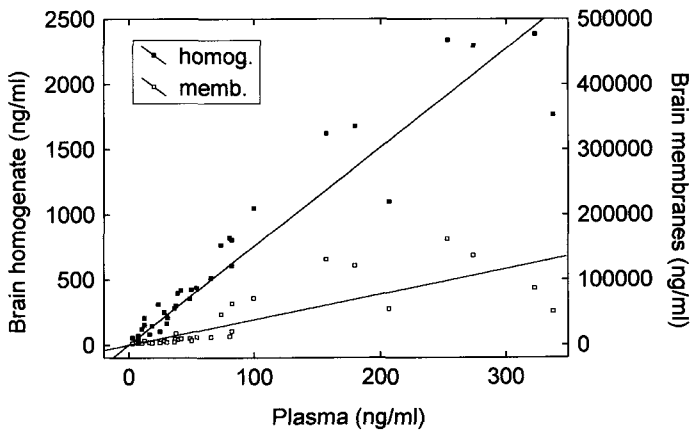


Figure 3. IMI+DMI concentrations in brain homogenate or brain membranes plotted vs blood plasma concentrations.

absolute values of IMI+DMI concentrations in the plasma and in RBC membranes (Fig. 2); the plasma and brain membranes (Fig. 3); brain homogenate (B_HOM) and brain or RBC membranes (Fig. 4), etc.

In an effort at quantitative assessment of the association between IMI+DMI concentrations in various samples, correlation coefficients were calculated for all pairs of concentrations measured in individual animals (Table 1). Statistically sig-

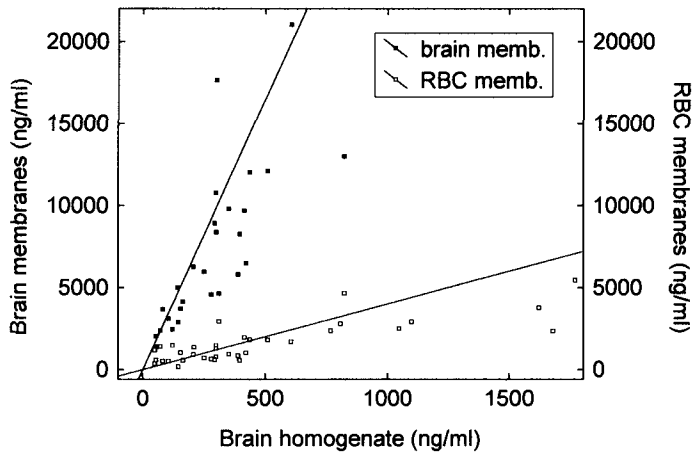


Figure 4. IMI+DMI concentrations in brain membranes or RBC membranes plotted vs concentrations in brain tissue homogenate (points at higher concentrations not shown).

Table 1. Correlation coefficients for all pairs of IMI+DMI concentrations measured in the plasma (R_PLA), haemoglobin (R_HEM), red blood cells (R_RBC), erythrocyte ghosts (R_GHO), RBC membranes (R_MEM), brain homogenate (B_HOM), brain cytosol (B_CYT), and brain membranes (B_MEM); $N = 40$

	R_HEM	R_RBC	R_GHO	R_MEM	B_HOM	B_CYT	B_MEM
R_PLA	0.954	0.975	0.949	0.883	0.955	0.811	0.806
R_HEM	1.000	0.971	0.974	0.827	0.890	0.690	0.696
R_RBC		1.000	0.977	0.899	0.950	0.813	0.791
R_GHO			1.000	0.907	0.921	0.762	0.750
R_MEM				1.000	0.911	0.846	0.779
B_HOM					1.000	0.917	0.922
B_CYT						1.000	0.950

correlation coefficient

nificant associations could be established, at a significance level lower than 0.001, between all paired concentrations measured.

As all the measured parameters correlate well, simple coefficients can be used for their mutual conversion (Table 2). Average values of ratios of concentrations (better than coefficients of linear regression) were employed to avoid overestimated values at higher concentrations. The values given in Table 2 show that IMI+DMI tend to accumulate heavily in the brain tissue (represented by B_HOM), particularly in brain membranes. The concentrations in brain tissue homogenate was 14.8

Table 2. Coefficients for IMI+DMI blood and brain concentration pairs. Calculated from ratios of concentrations measured in the plasma (R_PLA), red blood cells (R_RBC), erythrocyte ghosts (R_GHO), RBC membranes (R_MEM), brain homogenate (B_HOM), cytosol (B_CYT), and brain membranes (B_MEM). The concentrations R_MEM and B_MEM are shown in ng per ml of phospholipid membranes, while the other concentrations are expressed in ng per ml of brain tissue, red blood cells, plasma, haemoglobin, and cytosol. Mean \pm S.E.M., $N = 40$

x	R_PLA	R_RBC	R_GHO	R_MEM	B_HOM	B_CYT	B_MEM
R_PLA	1.00	1.66 (0.09)	2.43 (0.19)	0.0383 (0.0036)	0.121 (0.006)	1.90 (0.21)	0.00415 (0.00031)
R_RBC	0.620 (0.032)	1.00	1.38 (0.07)	0.0221 (0.0019)	0.0742 (0.0037)	1.11 (0.10)	0.00263 (0.00021)
R_GHO	0.504 (0.043)	0.794 (0.046)	1.00	0.0151 (0.0009)	0.0567 (0.0037)	0.849 (0.087)	0.00203 (0.00020)
R_MEM	36.2 (4.9)	65.1 (7.0)	72.6 (4.1)	1.00	4.17 (0.43)	63.1 (6.6)	0.166 (0.025)
B_HOM	9.06 (0.45)	14.8 (0.9)	20.7 (1.6)	0.314 (0.029)	1.00	14.1 (0.9)	0.0349 (0.0024)
B_CYT	0.736 (0.075)	1.16 (0.10)	1.53 (0.15)	0.0223 (0.0022)	0.0812 (0.0065)	1.00	0.00265 (0.00026)
B_MEM	324 (32)	488 (46)	684 (85)	10.9 (1.3)	35.8 (2.9)	494 (41)	1.00

coefficient, k ($y = k \cdot x$)
(S.E.M.)

times that in RBCs, and the concentrations in brain membranes were about 11 times those in RBC membranes.

The association between the blood concentrations and those in the brain tissue was analysed in more detail. As dependence on different identical variables may underlie an association between two variables, partial correlation coefficients were calculated. The analysis revealed a primary association between the concentrations in brain homogenate (B_HOM), blood plasma (R_PLA) and RBC membranes (R_MEM).

Multiple regression analysis made it possible to determine the best approximation of the parameter "antidepressant concentration in the brain tissue" using concentrations in blood samples. A significant association could be established between the concentrations in brain homogenate and that in the blood plasma plus

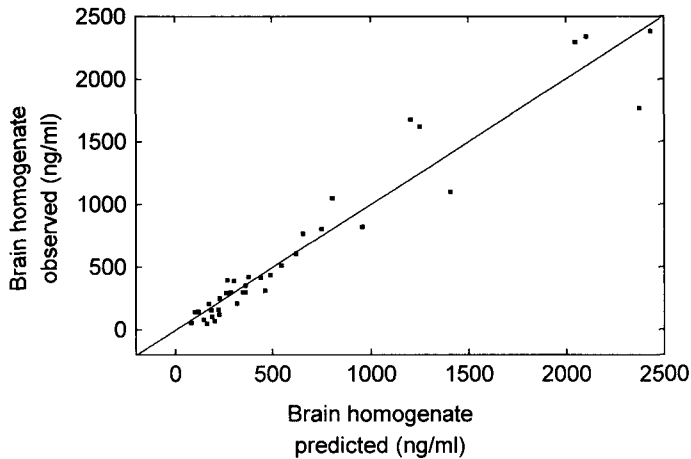


Figure 5. Observed and predicted IMI+DMI concentrations in rat brain tissue. Observed values were defined as the drug concentrations measured in brain homogenate from 40 rats receiving imipramine at doses of 4–38 mg/kg per day for a period of 7 days. Predicted values were calculated using equation (2) from concentrations measured in the blood plasma and in RBC membranes in the same group of experimental animals.

RBC membranes:

$$B_HOM = 5.16 \cdot R_PLA + 0.116 \cdot R_MEM \quad (2)$$

where IMI+DMI concentrations, B_HOM and R_PLA, are related to 1 ml of sample, and R_MEM is related to 1 ml of phospholipid membranes. Coefficients were calculated with the following standard errors: 5.16 ± 0.66 ($N = 40$, t -value=7.8, $p < 0.001$), 0.116 ± 0.030 ($N = 40$, t -value=3.9, $p < 0.001$). Equation (2) can be used within the range of plasma concentrations from 3 to 330 ng/ml. We obtained a very fair estimate of IMI+DMI concentrations in the rat brain derived from blood concentrations ($R = 0.96$, Fig. 5).

Discussion

The brain is the target organ of antidepressants. Factors affecting brain concentrations of drugs include lipid-water partition coefficients. In this study concentrations were measured in the plasma, RBCs, RBC membranes, haemoglobin, brain homogenate, brain membranes and cytosol after chronic administration of IMI.

Concentrations of antidepressants in membranes are much higher than in the plasma; this may be important for their effect on function of many membrane systems. The hypothesis of a role of the lipid bilayer in the mode of action of

tricyclic antidepressants is supported by many indirect observations (Moor et al. 1988; Bauer et al. 1990; Toplak et al. 1990; Fišar et al. 1991; Krulík et al. 1991b; Bhat and Block 1992).

To express the concentration of a drug in the membranes, it is more appropriate to use the concentration in the lipid bilayer instead of concentration in membrane suspension (Heirwegh et al. 1992). Therefore, we related IMI+DMI concentrations in RBC membranes and in brain membranes to the phospholipid contents of samples. Membrane concentrations were found to exceed plasma concentrations by one or two orders (Table 2), and to reach tens to hundreds $\mu\text{mol/l}$ at therapeutic plasma levels (Figs. 2, 3). The concentrations in brain membranes exceeded those found in the RBC membranes 10.9 times (Table 2, Fig. 4).

The present findings suggest that IMI+DMI concentrations in the brain are in direct relation to both plasma and RBC membranes concentrations. To estimate IMI+DMI concentrations in the brain tissue, the concentrations measured in blood components and multiplied using coefficients shown in Table 2 or, better still, equation (2), can be used. Regarding other antidepressants, these coefficients and associations can be quite easily determined using the procedure described in this paper.

The association between the IMI+DMI concentrations in the brain and those in the blood (the plasma, RBCs, RBC membranes, haemoglobin) was determined by partial correlation analysis and by multiple regression analysis. There exists a direct dependence of the brain tissue concentrations on the concentrations of IMI+DMI both in blood plasma and RBC membranes (Eq. 2). Although all paired concentrations measured correlate well (Table 1), there is no direct dependence of the brain concentrations on the concentrations in whole RBC or haemoglobin.

The above results may help clarify the fact that the plasma concentrations of antidepressants do not correlate well with the therapeutic effects of the drugs in some cases. An explanation for this finding may be that the concentrations in the brain tissue are determined both by the concentrations in the plasma and in the cellular membranes of blood elements. It can be hypothesized, that changes in the distribution of antidepressants between plasma and RBC membranes, e.g. due to alterations in the phospholipid composition of these membranes, may have an effect on the brain tissue concentrations, which then do not necessarily correlate well with the plasma concentrations. Changes in the phospholipid composition of membranes must be considered when studying the therapeutic effects of antidepressants.

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