Effect of Long-Term Administration of Antidepressants on the Lipid Composition of Brain Plasma Membranes

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Abstract. The connection between changes in lipid pattern in brain plasma membranes and long-term administration of therapeutically effective doses of antidepressants has not been sufficiently demonstrated so far. Therefore, we analyzed effect of antidepressants that differ in pharmacological selectivity on membrane lipid composition in the rat brain tissue. Laboratory rats were given desipramine, maprotiline, citalopram, moclobemide or lithium for a 4-week period. We observed a significant decrease in phosphatidylethanolamine representation after administration of maprotiline, citalopram and moclobemide when compared with controls. Membrane cholesterol content was decreased after desipramine administration and increased after citalopram or lithium treatment. Electroneutral phospholipids were decreased after the administration of all tested antidepressants except for desipramine. Decrease in phosphatidylserine was found following long-term administration of maprotiline or desipramine; relative representation of phosphatidylinositol was reduced after lithium treatment. Statistically significant negative correlation between cholesterol and electroneutral phospholipids was discovered. Membrane microviscosity evaluated by fluorescence anisotropy of membrane probes was only slightly decreased after desipramine and increased after citalopram administration. Hypothesis was supported that changes in brain neurotransmission produced by antidepressants could be, at least partially, associated with adaptive changes in membrane cholesterol and phospholipids.

Key words: Antidepressant — Phospholipid — Cholesterol — Plasma membrane — Rat brain

Introduction

Antidepressants are known to realize their primary biochemical actions through the binding to specific binding sites of neurotransmitter receptors, transporters

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or catabolizing enzymes. However, most of antidepressants belong to the cationic amphiphilic drugs group and interact more or less specifically with lipid bilayer also (Herbette et al. 1986; Seydel et al. 1994; Fišar et al. 2004). It results in changes of rotational and lateral diffusion, ordering and mutual interactions of membrane molecules, which can influence transmembrane signal transduction and play a part in effects of antidepressants. There is insufficient knowledge as regards the extent in which the accumulation of antidepressants in lipid part of brain cell membranes contributes to their therapeutic or adverse effects.

Phosphatidylcholine (PC), sphingomyelin (SM) and phosphatidylethanolamine (PE) are the most abundant lipids in the biological membranes; however, acidic phospholipids, such as phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylglycerol, phosphatidic acid and gangliosides, play a key role in direct activation of various enzymatic and receptor systems (Tsakiris and Deliconstantinos 1984; Rando 1988; Sandermann and Duncan 1991; Lee 2003). The role of cholesterol consist both in direct action on the function of some membrane proteins (Scanlon et al. 2001; Fielding and Fielding 2003; Pfrieger 2003) and in regulation of the membrane structure, lateral organization, free volume distribution and "fluidity" (Shinitzky 1984; Burger et al. 2000; Barenholz 2002). Cholesterol is most likely associated with PCs and SMs in the membranes (Ohvo-Rekilä et al. 2002).

A lot of abnormalities related to lipid homeostasis were described in depression (Maes et al. 1997; Kato et al. 1998); however, little is known about ability of antidepressants to induce alterations in the brain membrane phospholipid turnover at therapeutic drug concentrations. Phospholipid methylation in synaptic membranes or *in vivo* phospholipid administration can possibly influence receptor adaptation to chronic administration of antidepressants (Sulser et al. 1983; Racagni and Brunello 1984). Several papers reported that tricyclic antidepressants had a stimulatory effect on PS synthesis in cell cultures (Singh et al. 1992; Bobeszko et al. 2002); however, this effect was significant at relatively high drug concentrations. Many antidepressants, including tricyclic antidepressants and selective serotonin reuptake inhibitors, may induce excessive intracellular accumulation of different phospholipids (generalized phospholipidosis, phospholipid storage disorder) in cultured cells or in several organs of experimental animals. However, drug concentrations far from therapeutical plasma levels were used in these experiments (Honegger et al. 1983; Lüllmann-Rauch and Nassberger 1983; Stoffel et al. 1987; Xia et al. 2000). Organspecific changes in the phospholipid composition were observed in rats after the long-term administration of therapeutical doses of the desipramine, but no changes were found in whole brain (Moor et al. 1988). Changes in anisotropies of the superficial membrane layers and in lipid composition (PC/PE ratios) were observed following exposure of cultured human fibroblasts to high desipramine concentrations (Toplak et al. 1990). The synthesis of PI was inhibited *in vitro* by a great number of antidepressants; however, pharmacological significance of this inhibitory action is doubtful due to very high concentration of the drugs required (Li et al. 1988).

More data were reported on action of prophylactic drug lithium on membrane lipids. Data suggest that lithium exert both multiple effects on signal transduction and neuroprotective effects (Jope 1999; Manji et al. 1999). Lithium at therapeutic concentrations has been shown to have a strong inhibitory effect on arachidonic acid specific phospholipase A_2 (PLA_2) in the rat brain *in vivo*, i.e. it reduces turnover of the major *n*-6 polyunsaturated fatty acids in several brain phospholipids (Chang and Jones 1998). Brief chronic lithium administration to rats led to the subtle depressing the biosynthesis of PI as well as PE in the brain (Navidi et al. 1991). Likewise, lithium treatment induced small decreases (by several percent) in PS and PI brain levels (Pettegrew et al. 2001); it should be noted that even small changes in the quantity of these key membrane phospholipids could influence the function of many membrane proteins. No significant changes on the cholesterol/phospholipid ratio were observed in the synaptosomal plasma membranes after chronic administration of lithium (Lopez-Corcuera et al. 1988). Clinically relevant concentrations of lithium have been reported to affect membrane molecular dynamics both on the erythrocyte membrane surface (Pettegrew et al. 1987) and on the synaptosomal plasma membrane (Lopez-Corcuera et al. 1988).

Though the primary biochemical effects of antidepressants are described very precisely, their therapeutic effects are associated with adaptive cellular changes following their long-term (2–4 weeks) administration (Stahl 2000). All antidepressants have a common action on monoamine neurotransmission, leading to changes in gene expression in the neurons targeted by monoamine neurotransmitters. We suppose that this includes not only desensitization of neurotransmitter receptors but also regulation of the composition of brain lipids. The aim of our experiments was to determine how the long-term administration of various antidepressants affects the lipid composition of plasma membranes in rat brain. We decided to study the effect of five widely used antidepressants, desipramine (potent nonselective norepinephrine reuptake inhibitor), maprotiline (selective norepinephrine reuptake inhibitor), citalopram (selective serotonin reuptake inhibitor), moclobemide (reversible monoamine oxidase inhibitor), and lithium (mood stabilizer with various effects on signal transduction).

Materials and Methods

Chemicals and solutions

Water solutions of following concentrations of antidepressants were administered to rats: 2 mg/ml desipramine (Sigma-Aldrich Co., St. Louis, MO, USA), 2 mg/ml maprotiline (Ciba Geigy Ltd., CH-4002 Basle, Switzerland), 1 mg/ml citalopram (H. Lundbeck A/S, DK-2500 Copenhagen-Valby, Denmark), 5 mg/ml moclobemide (F. Hoffmann-La Roche Ltd., Basle, Switzerland) and 10 mg/ml lithium carbonate (Sigma-Aldrich Co., St. Louis, MO, USA). Sucrose buffer (0.32 mol/l sucrose, 10 mmol/l Tris HCl, 1 mmol/l $MgCl_2$, pH 7.4) with protease inhibitors (0.5 mmol/l phenylmethanesulfonyl fluoride, 0.5 mmol/l benzamidine HCl, 1 mmol/l EDTA,

2 mmol/l dithiothreitol) was used as grinding medium. Buffer was prepared one day in advance, it's pH was adjusted and following the filtration through 0.45 μm filter, it was stored at 4 °C. Dichloromethane/methanol (2 : 1, v/v) containing 0.25 % HCl (conc.) was used as an extraction mixture to remove lipids from membranes. Buffer A (120 mmol/l NaCl, 10 mmol/l KCl, 30 mmol/l Tris HCl, pH 7.4) and the membrane probes 1,6-diphenyl-1,3,5-hexatriene (DPH, Sigma-Aldrich Co., St. Louis, MO, USA) and 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH; Molecular Probes Inc., Eugene, OR 97402, USA) were used for the measurement of relative changes of plasma membrane microviscosity. DPH was dissolved in 6 mmol/l acetone solution, TMA-DPH in 3 mmol/l methanol solution, and these stock solutions were stored in a freezer. Fresh 6 μmol/l solutions of probes in buffer A were prepared for every measurement.

Laboratory rats

Specific pathogen-free Wistar strain of laboratory rats was used, total count of 45 males (15 in control group plus 5 groups by 6 animals) fed with standard diet under defined conditions (room temperature 22 °C, relative humidity 65 %, lighting 12 h/day). Keeping and treatment of laboratory animals conformed to the Declaration of Helsinki and was approved by the animal care committee of our Institution. Desipramine, maprotiline, citalopram, moclobemide and lithium carbonate were given with gastric tube once daily in doses of 10, 10, 5, 25 and 50 mg/kg *per* day, respectively, for the period of 4 weeks (period sufficient for realization of adaptive cellular changes related to therapeutic action of antidepressants). Administered doses were set according to human daily doses (75–150 mg/day for desipramine or maprotiline, 20–60 mg/day for citalopram, 100–400 mg/day for moclobemide, and 600–900 mg/day for lithium), but recalculated, supposing about 60 times lower body surface area of a rat. The weight of the rats was regularly monitored; initial weight was 180 ± 20 g, final 329 ± 27 g. The dosages were adjusted continuously according to the actual weight of a rat so that the dose *per* kg *per* day remained constant. The control group of rats was given water. The last dose was given one day before the rats were killed, which was done by exsanguinations from aorta under thiopental (Spofa, Czech Republic) anaesthesia. The whole brains were removed and immediately processed as described below.

Isolation of plasma membranes

Plasma membranes were isolated according to the method of Scott et al. (1993) with our modification. Briefly, after the weight was checked, whole brain was cut into pieces and placed in ice-cold sucrose buffer with protease inhibitors. Fine homogenization of the brain tissue was made in a glass homogenizer with teflon piston. Homogenate was replenished with grinding medium to 6 ml and centrifuged at $280 \times g$ for 10 min at 4 °C. Supernatant was attentively removed so that 1/3 of total volume was left down to avoid stirring up of pellet. Supernatant was stored in an ice-cold bath. Pellet was re-suspended in 5 ml of the sucrose buffer with protease inhibitors, centrifuged at $280 \times g$ for 10 min at 4 °C, and supernatant was attentively

removed. Both supernatants were put together and centrifuged at 20,000 × *g* for 20 min at 4 °C. Supernatant was decanted and pellet was homogenized using Ultra-Turrax (Janke & Kunkel, IKA Werk) with 5 ml of icy distilled water. Sample was centrifuged at 5000 × *g* for 20 min at 4 °C. Supernatant was stored, pellet was again turraxed with 5 ml of icy distilled water and sample was centrifuged (5000 × *g*, 20 min, 4 °C). Supernatants were put together and centrifuged (50,000 × *g*, 20 min, 4 °C). Pellet was re-suspended in 1 ml of buffer A and sample was rapidly frozen and stored at −50 °C.

Enrichment of the samples with plasma membranes was monitored during their isolation by determining ecto-5′-nucleotidase activity (Mitchell and Hawthorne 1965). Enrichment of samples by plasma membranes was about threefold when compared with crude membrane fraction of the brain homogenate. Protein concentrations were measured by Lowry method (Lowry et al. 1951). Lipidic phosphorus was assessed by Bartlett method (Bartlett 1959) following sample combustion with perchloric acid (60 min, 180 °C).

Isolation of membrane lipids

Modified Folch et al. (1957), Koul and Prasad (1996) methods were used to isolate membrane lipids. Briefly, samples were homogenized in Ultra-Turrax and centrifuged for 10 min at 20,000 × *g*. Supernatant was discarded and pellet was diluted in dichloromethane/methanol mixture (2 : 1, v/v, acidified by 0.25 % HCl), which volume was 20 times the original pellet volume. Test tubes content was gently stirred in dry bath at room temperature for 15–30 min under nitrogen atmosphere. Denatured proteins were removed by filtration through a mull and the filtrate (approximately 5 ml) was transferred into a calibrated test tube, circa 1 ml (20 volume percent) of water was added and the mixture was stirred. Phases were separated by 5-min centrifugation at 1000 × *g*. Top layer was carefully disposed off and the pure bottom layer containing most of lipids was transferred into labelled pre-weighted vial and evaporated under nitrogen. The vial was then weighted again and stored in freezer under nitrogen atmosphere.

Membrane lipid analysis

Membrane lipids were analyzed by two-dimensional thin-layer chromatography (TLC) on glass plates. Epicoprostanol (30 percentage by weight) was added to sample as internal standard for determination of cholesterol using gas chromatography. The samples in chloroform/methanol (2 : 1, v/v) solvent were spotted (40 μl, 10 mg/ml) on TLC plates and allowed to develop for 1 h and 20 min in the first dimension (chloroform/methanol/ammonium hydroxide/water, 70 : 25 : 4 : 1, v/v/v/v) and then for 1 h and 30 min in the second dimension (chloroform/methanol/acetone/acetic acid/water, 70 : 12.5 : 17.5 : 10 : 4.5, v/v/v/v/v). Lipids were visualized by exposing the plates to iodine vapours; fractions were scraped off for phosphorus (Bartlett 1959) and cholesterol assessment (Tvrzická et al. 1991).

Method of fluorescent probes

Relative changes in membrane fluidity were assessed by the method of fluorescent probes with membrane probes DPH and TMA-DPH (Prendergast et al. 1981; Plášek and Jarolím 1987; Lakowicz 1999). Fresh 6 μmol/l DPH or TMA-DPH solution in buffer A was mixed with aliquots of buffer A and membrane suspension, so that the resultant concentration of fluorescent probe was 2 μmol/l, and the phospholipid concentration was about 100 μmol/l. Following 60 min incubation at 37°C, the fluorescence polarization was measured on SLM 4800 spectrofluorometer using an excitation wavelength of 360 nm and an emission wavelength of > 405 nm. The fluorescence anisotropy was then calculated; increase in fluorescence anisotropy can be interpreted as an increase in the extent of probe movement restriction in the anisotropic membrane environment.

Data analysis

Data are expressed as the arithmetic means. Standard deviation (S.D.) was calculated to characterize group variability. Hypothesis testing was performed using analysis of variance (ANOVA), followed by post hoc Duncan's multiple range test. Spearman R (nonparametric alternative to the Pearson product-moment correlation coefficient) was used to quantify relation between two quantitative parameters. Normality of distribution has been verified by the Shapiro–Wilk's W test. Statistical analyses were performed with the statistical package Statistica (StatSoft Inc., Tulsa, USA).

Results

Male Wistar rats were dosed for four weeks with desipramine, maprotiline, citalopram, moclobemide and lithium (5 groups by 6 animals) and compared with control group ($n = 15$). During the course of experiment, the weight of the rats was regularly monitored in order to calculate antidepressant dosage and to determine its influence on the weight of laboratory rats. The final body weight (329 ± 27 g, $n = 45$) did not differ between all groups except for maprotiline (343 ± 17 g) and moclobemide (308 ± 30 g, $p = 0.047$) treated rats. The relative weight gain ($85 \pm 20\,\%$ of initial body weight, $n = 45$) was found significantly increased after citalopram ($100 \pm 15\,\%$, $p = 0.0056$) or lithium ($98 \pm 12\,\%$, $p = 0.0078$) treatment when compared to controls ($69 \pm 7\,\%$).

Both the whole brain weight (1.91 ± 0.16 g, $n = 45$) and the relative brain weight ($0.593 \pm 0.047\,\%$, $n = 45$, related to final body weight) did not differ significantly between all groups.

Lipid composition of plasma membranes in a rat brain

Total lipid (TL) was determined by weighing dried lipid extract, total phospholipid (PL) weight was determined from the phosphorus content in lipid extract, and cholesterol (CH) was measured by gas chromatography. We did not find statistically significant changes in ratios of these parameters (Fig. 1). The mean ratios

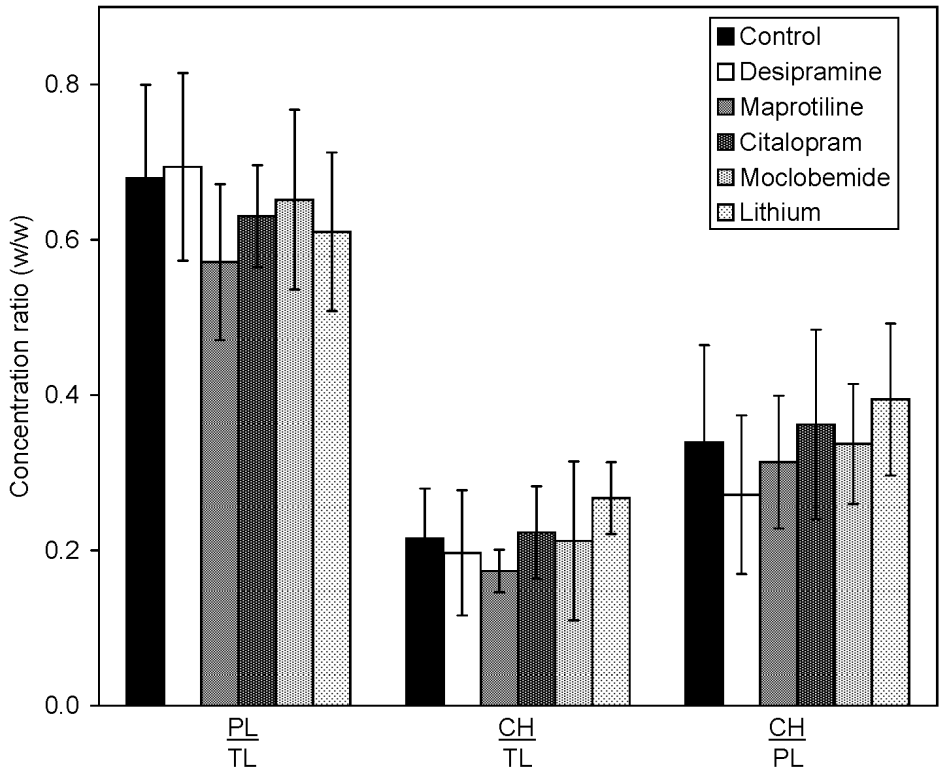

Figure 1. Ratios of weight to weight concentrations of total lipids (TL), total phospholipids (PL) and cholesterol (CH) in rat plasma membranes following 4-week administration of antidepressants. Values are means ± S.D. The means were calculated from 15 values in case of control group and from 6 values in all other groups. Values after antidepressant treatment are not significantly different from controls; determined by ANOVA and post hoc Duncan's test.

(weight/weight) in the control group were found PL/TL = 0.68 ± 0.12, CH/TL = 0.22 ± 0.06, and CH/PL = 0.34 ± 0.12. A decrease both in PL/TL (by 16%) and CH/TL (by 20%) ratios were found after the treatment with maprotiline. The CH/PL ratio was decreased by 20% after desipramine administration. The increase both in CH/TL (by 24%) and CH/PL (by 16%) ratios were observed after lithium administration also.

To interpret the changes in the relative representation of individual lipids in brain plasma membranes due to administration of antidepressants, we used the sum of CH+PL molar concentrations as the 100% value (Table 1). Low values of relative representation of PE, which was observed in all groups, are caused by a method used in the isolation of membrane lipids, i.e. by acidification of ini-

Table 1. Relative representation of molar concentrations of individual phospholipids or cholesterol in plasma membranes isolated from the brain of laboratory rats following 4-week administration of various antidepressants (in relation to total molar concentration of phospholipids and cholesterol)

Group	PC	PE	SM	PI	PS	CH
Controls	0.208 ± 0.057	0.042 ± 0.017	0.075 ± 0.016	0.044 ± 0.012	0.043 ± 0.018	0.369 ± 0.102
Desipramine	0.202 ± 0.055	0.036 ± 0.011	0.076 ± 0.024	0.038 ± 0.012	0.033 ± 0.018	0.331 ± 0.101
Maprotiline	0.167 ± 0.038	0.024 ± 0.003 *	0.065 ± 0.020	0.036 ± 0.011	0.025 ± 0.012	0.373 ± 0.056
Citalopram	0.181 ± 0.026	0.024 ± 0.005 *	0.061 ± 0.014	0.041 ± 0.006	0.047 ± 0.017	0.402 ± 0.078
Moclobemide	0.186 ± 0.026	0.024 ± 0.005 *	0.068 ± 0.010	0.039 ± 0.014	0.048 ± 0.021	0.374 ± 0.108
Lithium	0.166 ± 0.032	0.031 ± 0.020	0.063 ± 0.010	0.033 ± 0.011	0.040 ± 0.009	0.419 ± 0.098

Values are means ± S.D. The means were calculated from 15 values in case of control group and from 6 values in all other groups. PC, phosphatidylcholine; PE, phosphatidylethanolamine; SM, sphingomyelin; PI, phosphatidylinositol; PS, phosphatidylserine; CH, cholesterol; PL, total phospholipids. Sum of (CH+PL) molar concentrations was used as the 100% value. Low values of relative representation of PE are caused by a method used in the isolation of membrane lipids. Marked values are significantly different from controls at * $p < 0.05$; determined by ANOVA and post hoc Duncan's test.

tial dichloromethane/methanol mixture. We observed a significant decrease in PE representation after administration of maprotiline, citalopram and moclobemide compared to control group. No statistically significant changes were found both in other phospholipids and in cholesterol representation although it seemed that there was a decrease in PC, PE and SM after the administration of all tested antidepressants except for desipramine. PS was nonsignificantly decreased after maprotiline and desipramine, PI was decreased after lithium treatment. Cholesterol representation was decreased after desipramine and increased after citalopram or lithium administration compared to controls.

Other ratios of molar concentrations of membrane lipids were calculated to discover a relation between CH, PC, PE, SM, PI and PS. Data had shown no significant decrease in SM/PC ratio after citalopram administration (0.333 ± 0.036, $n = 6$) in comparison with controls (0.375 ± 0.075, $n = 15$, $p = 0.348$). Statistically significant increase was found in ratio of main electroneutral phospholipids (PC+PE+SM) and sum of individual phospholipids molar concentrations (SUM = PC+PE+SM+PI+PS) after treatment with desipramine (0.820 ± 0.048, $n = 6$, $p = 0.036$) or maprotiline (0.814 ± 0.037, $n = 6$, $p = 0.05$) in comparison with citalopram administration (0.749 ± 0.058, $n = 6$). It is exactly the opposite for (PI+PS)/SUM ratio (Fig. 2). It seems that representation of PS is responsible for this effect because statistically significant decrease was found in ratio of PS/SUM after treatment with desipramine (0.082 ± 0.040, $n = 6$, $p = 0.038$) or maprotiline (0.080 ± 0.032, $n = 6$, $p = 0.034$) in comparison with citalopram administration (0.135 ± 0.048, $n = 6$).

Plasma membranes microviscosity

The results for DPH and TMA-DPH probes in membranes isolated from the brain are shown in Table 2. There were no significant changes in fluorescence anisotropy of DPH or TMA-DPH after antidepressant administration in comparison with controls. Relatively most change in fluorescence anisotropy of DPH was observed after the desipramine administration (decrease by 1.5 %). Statistically significant increase in fluorescence anisotropy of DPH was found after treatment with citalopram in comparison with desipramine administration ($p = 0.018$) and in fluorescence anisotropy of TMA-DPH after treatment with citalopram ($p = 0.011$) or moclobemide ($p = 0.046$) compared with desipramine.

Mutual relations between variables

Mutual relations between variables given in Table 1 were tested by means of a correlation coefficients matrix (Table 3). Spearman correlations that were calculated from 45 cases consist of controls plus antidepressants administered rats. In accordance with what had been expected, a significant positive correlation between the relative representations of individual phospholipids and their sum was found. A significant negative correlation between the relative representation of CH and electroneutral phospholipids (PC, PE, and SM) was discovered. Correlations between CH and acidic phospholipids (PS, PI) were negative but statistically no

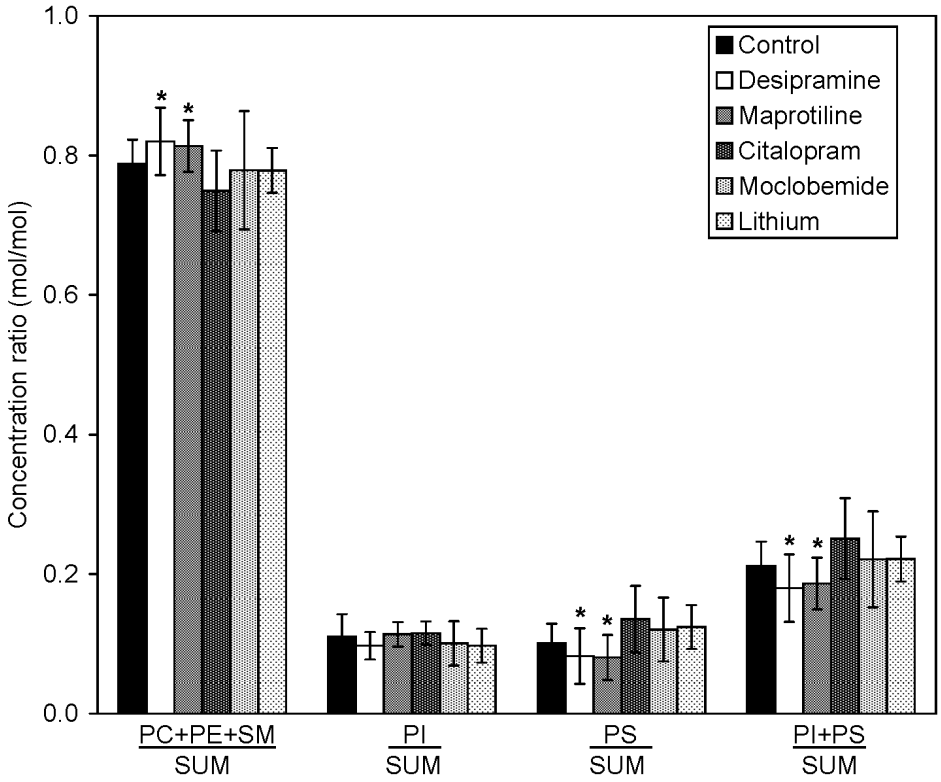

Figure 2. Relative representation of molar concentrations of main electroneutral phospholipids (PC+PE+SM), phosphatidylinositol (PI), phosphatidylserine (PS), and PI+PS, respectively, in plasma membranes isolated from rat's brains following 4-week administration of various antidepressants (in relation to sum of individual phospholipids molar concentrations; SUM = PC+PE+SM+PI+PS). Values are means ± S.D. The means were calculated from 15 values in case of control group and from 6 values in all other groups. Marked values are significantly different from citalopram-treated rats at * $p < 0.05$; determined by ANOVA and post hoc Duncan's test.

significant. Fluorescence anisotropy both in DPH and TMA-DPH probe did not correlate with other variables due to very small changes in this parameter after antidepressant administration; there was only a significant mutual relation between DPH and TMA-DPH fluorescence anisotropy characterized by correlation coefficient $R = 0.782$ ($p < 0.0001$).

Discussion

Our study is a contribution to the understanding the role of membrane lipids in the treatment of depressive disorder. Disturbances in the lipid metabolism in depression

Table 2. Fluorescence anisotropy of DPH and TMA-DPH in plasma membranes isolated from rat's brains following 4-week administration of various antidepressants

Group	DPH	TMA-DPH
Controls	0.2074 ± 0.0036	0.2547 ± 0.0023
Desipramine	0.2043 ± 0.0020	0.2529 ± 0.0025
Maprotiline	0.2055 ± 0.0017	0.2540 ± 0.0022
Citalopram	0.2090 ± 0.0038 *	0.2570 ± 0.0031 *
Moclobemide	0.2079 ± 0.0025	0.2560 ± 0.0019 *
Lithium	0.2054 ± 0.0016	0.2534 ± 0.0021

Values are means ± S.D. The means were calculated from 15 values in case of control group and from 6 values in all other groups. DPH, 1,6-diphenyl-1,3,5-hexatriene; TMA-DPH, 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene. Values after antidepressant treatment are not significantly different from controls. Marked values are significantly different from desipramine-treated rats at * $p < 0.05$; determined by ANOVA and post hoc Duncan's test.

Table 3. Relation between relative representation of molar concentrations of phospholipids and cholesterol in brain plasma membranes (Table 1) determined as correlation coefficients (Spearman R)

	PE	SM	PI	PS	PI+PS	SUM	CH
PC	0.523***	0.701***	0.520***	0.455**	0.569***	0.921***	−0.719***
PE	1.000	0.548***	0.297*	0.302*	0.401**	0.623***	−0.315*
SM		1.000	0.457**	0.245	0.386**	0.783***	−0.404**
PI			1.000	0.434**	0.829***	0.632***	−0.230
PS				1.000	0.841***	0.603***	−0.276
PI+PS					1.000	0.722***	−0.270
SUM						1.000	−0.573***

Spearman correlations between each pair of variables were calculated from 45 cases (controls plus antidepressants administered rats). PC, phosphatidylcholine; PE, phosphatidylethanolamine; SM, sphingomyelin; PI, phosphatidylinositol; PS, phosphatidylserine; SUM, sum of individual phospholipid molar concentrations (PC+PE+SM+PI+PS); CH, cholesterol. Marked correlations are significant at * $p < 0.05$, ** $p < 0.01$ or *** $p < 0.001$.

and restoration of balance by antidepressants are supposed by many hypotheses of affective disorders. However, the connection between changes in lipid pattern in brain plasma membranes and long-term administration of therapeutically effective doses of antidepressants has not been sufficiently demonstrated so far.

In this study, hypothesis was tested that the changes in the lipid composition of plasma membranes from brain could be included in the common adaptive effect of long-term administration of antidepressants. We investigated the effects of five an-

tidepressants (that differ in primary pharmacological selectivity) on the cholesterol and phospholipid composition and "microviscosity" of brain membranes. Antidepressants were given to laboratory rats for the period of 28 days, which is sufficient for realization of adaptive cellular changes owing to drugs. Changes in the length and saturation of acyl chains of membrane phospholipids were not measured in our study and interpretation of results is aggravated by the facts that the biochemical response of the rat after long-term administration of antidepressants need not be equal to the response in man.

Plasma membranes isolated from the whole brain of experimental animals were analyzed. Unfortunately, there is no single procedure that results in the quantitative recovery of all membrane lipids, so the absolute values of ratios determined can be shifted. We acidified the initial dichloromethane/methanol mixture for the recovery of acidic phospholipids, but the acidity leads to cleavage of PE plasmalogen. We did not solve this problem because the aim of our study was the determination of relative changes in lipid composition in membrane lipids after long-term administration of antidepressants and because lipids were extracted by the same technique for all groups. The results summarized in Table 1 and Fig. 2 showed that long-term administration of antidepressants had influence on lipid composition of cell membranes in the brain. However, the variability of individual results, as reflected by S.D., is rather high, which limits the statistical significance. The reason for the high variability is not known, as both non-normally distribution of values and dietary or seasonal variations can be ruled out in our study.

We have demonstrated that treatment with antidepressants evidently influenced the composition of lipid classes in the brain plasma membranes. Maprotiline, citalopram and moclobemide significantly decreased PE representation when compared to control group (Table 1). Although it is known that some antidepressants stimulate PS synthesis (Singh et al. 1992; Bobeszko et al. 2002) we did not observe any significant increase in PS in the brain plasma membranes (Table 1). Statistically significant decrease in ratio PS to sum of individual phospholipids was found after treatment with desipramine or maprotiline when compared with citalopram-treated group (Fig. 2).

The decrease in relative representation of electroneutral phospholipids was found systematically decreased after administration of all tested antidepressants except for desipramine; reduction of the conversion of PS to PE by amphiphilic cationic antidepressants (Kanfer and McCartney 1993) could participate in this effect.

No statistically significant but apparent decrease in cholesterol representation in brain membranes after long-term administration of desipramine and the increase in cholesterol after citalopram or lithium (Table 1) may be of great importance, as cholesterol strongly affects the properties of lipid bilayer and function of some membrane transporters, including serotonin transporter (Scanlon et al. 2001).

Based on these findings, we can assume that the effects of lithium and antidepressants differing in pharmacological selectivity could be related partly to the changes of cholesterol and phospholipids content in cell membranes and hence

resulting changes in the membrane proteins (e.g. serotonin or norepinephrine receptors and transporters) function. This cell membrane impact may be brain specific due to the fact that the concentration of antidepressants in the brain significantly exceeds their concentration in the periphery (Fišar et al. 1996). The opposite effects of desipramine administration in comparison with citalopram or with other tested antidepressants could be related to a nonspecificity of desipramine biochemical action.

Method of fluorescent probes with hydrophobic DPH and amphiphilic TMA-DPH membrane probes was used to determine relative changes in structural and physicochemical properties of specific membrane regions following long-term administration of various antidepressants. The rotational flexibility of DPH or TMA-DPH within the membrane expressed as steady-state fluorescence anisotropy gives information on the surrounding of the lipid environment of the probes (Lentz 1993). TMA-DPH showed fluorescence anisotropy values that were significantly higher with respect to DPH, suggesting a localization of the fluorescent portion of TMA-DPH in a more ordered region of membrane. Surprisingly, DPH and TMA-DPH fluorescence anisotropy measurements in plasma membranes isolated from the brain did not show any marked changes when compared to controls except for desipramine effect (Table 2).

Decrease in the fluorescence anisotropy after the desipramine administration was observed in usage of both DPH and TMA-DPH probe. It emerges from *in vitro* experiments (Sanganahalli et al. 2000) that predominant cause of the desipramine effect on membrane dynamic is not direct influence of drug on membrane structure. This result can be interpreted as a change in membrane dynamic properties both in the hydrophobic core of the membrane, where the DPH probe is localized (Kaiser and London 1998), and in the membrane/water interface, which is the target of TMA-DPH probe (Prendergast et al. 1981). Fluorescence anisotropy of both DPH and TMA-DPH was slightly increased after citalopram administration (Table 2). This increase was statistically significant when compared to desipramine administration, which could be related to the opposite effect of drugs on membrane cholesterol and/or phospholipids (Table 1). However, the increase in relative cholesterol content after lithium administration (Table 1) was not expressed in the expected increase in fluorescence anisotropy. Our results do not clearly indicate the reason for this fact.

Overall, the changes in steady-state fluorescence anisotropy of DPH or TMA-DPH produced by antidepressants cannot be assessed as very distinct. Accordingly, there are no substantial changes in dynamic properties of brain plasma membranes. However, both the time-resolved fluorescence spectroscopy and fatty acid analysis is required for detailed study of the effect of long-term treatment with antidepressants on membrane dynamic properties.

Long-term administration of antidepressants has rather small effect on relative representation of cholesterol and individual phospholipids in brain plasma membrane; the structure and dynamic properties of the brain plasma membrane does not show significant impairment compared to controls. Possible changes in cell

membranes fluidity could be considered as non-specific signs of a depressive disorder even in the case they were registered during the administration of antidepressants. We found in previous experiments that the increase in limiting permeability of the platelet membranes for serotonin could be included in the common adaptive effect of the long-term administration of antidepressants that differ in pharmacological selectivity (Fišar et al. 2005). Our results indicate that increased activity of serotonin transporter is caused rather by changes in cholesterol-transporter or phospholipid-transporter interactions, then by changes in membrane microviscosity.

It is difficult to interpret relation between changes in the composition brain lipids and therapeutic effects of antidepressants, because membrane lipids affect great number of cellular processes resulting in very different physiological effects. Nevertheless, our results support hypothesis about the role of adaptive changes of membrane cholesterol and phospholipids in mechanisms of action of antidepressants.

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