Effect of Pharmacologically Selective Antidepressants on Serotonin Uptake in Rat Platelets

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Abstract. We tested a hypothesis that a long-term administration of antidepressants acting through different primary biochemical mechanisms is associated with changes in the platelet serotonin (5-hydroxytryptamine, 5-HT) transport. Laboratory rats were administered norepinephrine reuptake inhibitors (desipramine, maprotiline), selective 5-HT reuptake inhibitor (citalopram), reversible monoamine oxidase inhibitor (moclobemide), and lithium (inositol monophosphatase inhibitor among others) during a 4-week period. Apparent kinetic parameters of platelet 5-HT transport were analyzed. Significant decrease in apparent Michaelis constant (K_M) was found after the administration of all tested antidepressants except for desipramine. There was certain increase in maximal velocity (V_{max}) values following the administration of desipramine, maprotiline, and citalopram; however, the all V_{max} changes were not significant. V_{max}/K_M ratio representing limiting permeability at low extracellular concentrations of 5-HT was systematically increased in all the tested drugs, but significant changes were occurred only in maprotiline- and citalopram-treated rats. Adaptive changes in platelet 5-HT transport induced by citalopram were opposite to the acute inhibitory effect of this drug on 5-HT transporter activity. An increase in limiting membrane permeability for 5-HT could be included in the common adaptive effect of the long-term administration of antidepressants that differ in pharmacologic selectivity.

Key words: Antidepressant — Serotonin uptake — Platelets

Introduction

The mechanisms of antidepressants action that are linked to their therapeutic effects are not sufficiently explained. Though the primary biochemical effects of these drugs are described very precisely, their therapeutic effects are associated with adaptive cellular changes following their long-term (several weeks) administration. Therefore it is difficult to decide, which cellular changes are actually responsible for

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therapeutic effects. Important group of antidepressants comprises of drugs primarily inhibiting reuptake of neurotransmitters from the synaptic cleft, particularly serotonin (5-hydroxytryptamine, 5-HT) and/or norepinephrine (NE) reuptake inhibitors (Owens 2004). However, also drugs with different primary mechanisms of action (monoamine oxidase inhibitors, 5-HT_{1A} receptors agonists, 5-HT_2 receptor or α_2-adrenoceptor antagonists, selective blockers of presynaptic dopamine receptors, etc.) are effective in the treatment of affective disorders (Stahl 2000). Moreover, mood disorders are classically treated with lithium, an ion whose mechanism of action is not fully understood. Lithium directly inhibits inositol phosphate metabolism by inhibition of inositol monophosphatase and inhibits glycogen synthase kinase-3 (Harwood and Agam 2003); a number of other signal transduction pathways are indirectly affected by lithium (Stahl 2000). There is general agreement that the clinical effect of antidepressants is caused by their ability to induce adaptive changes of the monoaminergic neurotransmitter systems (Manji 1992; Lachman and Papolos 1995; Duman et al. 1997; Duman 2002). Additional research is necessary to determine final common pathway for different antidepressants in specific brain regions.

There is evidence that pharmacologically selective antidepressants affect transmission mediated both by the targeted neurotransmitter and by the other neurotransmitters (Lucki and O'Leary 2004). Some early hypotheses of depression proposed that there are interactions between the serotonergic and noradrenergic systems (Caldecott-Hazard et al. 1991), or serotonergic and dopaminergic systems (Bonhomme and Esposito 1998). However, interconnectivity between 5-HT system and other neurotransmitter systems is not known sufficiently.

Based on findings of the presence of identical high affinity serotonin transporter (SERT) in the plasma membranes of neurons and platelets (Lesch et al. 1993) and on the similarities of platelets with 5-HT nerve terminals, particularly for the mechanism for 5-HT uptake, storage and release as well as 5-HT_{2A} receptor binding characteristics (Tuomisto et al. 1979; Stahl 1985; Murphy 1990; Maes and Meltzer 1995), it is believed that platelet 5-HT uptake can be used to assess brain serotonergic function. The SERT plays a pivotal role in maintaining of serotonergic function by regulation of the 5-HT level in the synaptic cleft (Haase et al. 2001). This plasma membrane transport carrier is given a lot of attention in 5-HT hypotheses of affective disorders, because the primary biochemical effect of some antidepressant drugs including today's most commonly used antidepressants, selective 5-HT reuptake inhibitors (SSRI), is SERT inhibition. The parameters of kinetics of 5-HT platelet uptake are considered as relevant in reflecting changes in the function of brain 5-HT system (Slotkin et al. 1986; Lesch et al. 1993; Bianchi et al. 2002). There is considerable evidence that depression and suicide may be associated with alterations in platelet 5-HT parameters (Langer and Schoemaker 1988; Mellerup and Langer 1990; Pandey et al. 1995; Franke et al. 2000), thus making platelets a non-invasive model for study of the mechanism of action of antidepressants. In recent years, peripheral parameters of serotonergic transmission, such as plasma and platelet 5-HT levels (Ortiz et al. 1988), have been used to examine the

mechanism of action of SSRIs and to predict the clinical response in depression (Bakish et al. 1997; Bourdeaux et al. 1998; Alvarez et al. 1999a; Blardi et al. 2002; Castrogiovanni et al. 2003; Maurer-Spurej et al. 2004).

In platelets, 5-HT is stored in dense-core granules (reserve pool) and shows a slow turnover. In contrast, extracellular plasma 5-HT reveals a rapid turnover and is pharmacologically active (Ortiz et al. 1988). Cationic amphiphilic drugs might accumulate in platelets, inhibit their functions and liberate in part 5-HT (Turčáni et al. 1982; Jančinová et al. 1996; Nosáľ and Jančinová 2002). It was demonstrated that therapeutic concentrations of the SSRI as well as the selective NE reuptake inhibitors decrease platelet and whole blood 5-HT concentrations (Alvarez et al. 1999b; Javors et al. 2000; Ashina et al. 2004). In contrast, 5-HT platelet content was significantly increased following moclobemide treatment (Lestra et al. 1998). There have been several review articles indicating that therapeutic effects of lithium can be related to its enhancements of serotonergic brain function (Meltzer and Lowy 1987; Shiah and Yatham 2000; Pandey et al. 2003). However, there is conflicting evidence with regard to the effect of lithium therapy on platelet 5-HT uptake. Lithium-induced changes at the level of 5-HT uptake in platelets were not correlated with concomitant variations in platelet 5-HT content (Glue et al. 1986; Poirier et al. 1988; Artigas et al. 1989).

The binding parameters need not sufficiently reflect changes in function of SERT because binding sites may be masked or reversible sequestered. Functional parameters of SERT must be measured. 5-HT uptake is a saturable carrier-mediated endergonic transport process (active transport) obeyed simple Michaelis–Menten kinetics with a maximal (limiting) velocity (V_{max}) and apparent Michaelis constant (K_M) (defined as extracellular 5-HT concentration required for transport velocity equal to $V_{max}/2$). The K_M can be related to the reciprocal value of affinity constant of binding site to the 5-HT. The uptake activity of SERT has been suggested to be controlled through endogenous pathways, e.g. by phosphorylation/dephosphorylation and/or by trafficking to specific plasma membrane subdomains (Blakely et al. 1998; Ramamoorthy and Blakely 1999; Haase et al. 2001). Effect of 5-HT reuptake inhibitors on SERT activity have been intensively studied; however, there is little known about the effect of long-term administration of pharmacologically different antidepressants on adaptive changes in transmembrane 5-HT transport.

It is hypothesised that the SERT activity is poised on by cellular mechanisms which are disturbed during depression and returned to the normal function following long-term antidepressant treatment. This mechanism may include the changes both in the expression of SERT and in the state of actin cytoskeleton (Sakai et al. 2000), cholesterol (Scanlon et al. 2001) and structure and composition of lipid bilayers (Block and Edwards 1987; Sheridan and Block 1988).

For all of the above reasons, it is important to determine if antidepressants that differ in pharmacologic selectivity produce a common *in vivo* effect on SERT activity. The aim of the study was to determine how the long-term administration of various antidepressants could affect active transmembrane transport of 5-HT in

platelets. To eliminate effects of depression on SERT activity and to avoid treatment of healthy human volunteer's with antidepressants, we studied the effect of long-term administration of selected antidepressants on the parameters of platelet 5-HT uptake in the blood of laboratory rats. The results were compared with acute *in vitro* action of antidepressants on the platelet 5-HT uptake in untreated rats. Potent nonselective NE reuptake inhibitor (desipramine), selective NE reuptake inhibitor (maprotiline), SSRI (citalopram), reversible monoamine oxidase inhibitor (moclobemide), and lithium were used in our study.

Materials and Methods

Chemicals and solutions

Water solutions of drugs 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). Anticoagulant-citrate-dextrose (ACD) solution (0.8% citric acid, 2.2% trisodium citrate, 2.4% glucose, pH 4.3) was used as anticoagulant. Platelets were incubated in modified Krebs–Ringer (KR) bicarbonate physiologic solution without Ca^{2+} (118 mmol/l NaCl, 4.7 mmol/l KCl, 1.2 mmol/l KH_2PO_4, 1.2 mmol/l $MgSO_4.7H_2O$, 25 mmol/l $NaHCO_3$, 11.1 mmol/l glucose; percolating with 95% O_2 and 5% CO_2 gas mixture was used during the preparation to saturate solution with oxygen, pH 7.4). 8 μmol/l tritium-labelled 5-HT ([^{3}H]5-HT) solution with specific activity of approximately 25 kBq/ml was prepared by mixing KR buffer with 1 mmol/l unlabelled (cold) 5-HT (5-hydroxytryptamine creatinine sulphate, Sigma) and [^{3}H]5-HT stock solution (specific activity of 500 to 1000 GBq/mmol, radioactive concentration 37 MBq/ml, radiochemical purity by high performance liquid chromatography >97%, Amersham Pharmacia Biotech UK Ltd., England). *In vitro* effect of antidepressants on platelet 5-HT transport was measured using 20 μmol/l stock solutions of desipramine, maprotiline, citalopram or moclobemide, and 4 mmol/l stock solution of $LiCO_3$ (all in KR solution).

Laboratory rats and blood collection

Specific pathogen-free Wistar strain of laboratory rats was used, total count of 49 males (19 in control group plus 5 groups by 6 animals) fed with standard diet under defined conditions. Keeping and treatment of laboratory animals conformed to the Declaration of Helsinki and was approved by the animal care committee of our Institution. Drugs were administered by gastric tube once a day for a total of 28 days. Administered doses were set according to human daily doses, 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*

kilogram *per* day remained constant. Table 1 shows the dosages of administered drugs. Control group of rats was administered by water. The last dose was administered one day before the rats were killed, which was done by exsanguinations from aorta under thiopental anaesthesia using plastic syringe. Blood samples (6 ml) were anticoagulated with ACD solution (1.5 ml), stirred and centrifuged in plastic test tubes at $200 \times g$, 20 ℃ for 15 min. 2 ml of platelet rich plasma (PRP) was transferred into a plastic container, platelets were counted under light microscope and the PRP concentration was adjusted to 200×10^9 platelets/l with KR solution. The 5-HT transport was assayed in intact platelets using modified Tuomisto et al. (1979) and Meltzer et al. (1981) methods as described below. Analyses for the 5-HT uptake were performed within 3 h after blood collection.

Our blood sample withdrawal technique might be controversial; both thiopental anaesthesia and taking blood from aorta with a syringe may pre-activate platelets. Very light anaesthesia (during normal oxygen saturation) and taking blood *via* plastic catheter (Van Den Berg et al. 1987; Kitamura et al. 2001; Dordoni et al. 2004) is recommended for these experiments. Although we did not proof whether the sampling procedure activates platelets, we suppose that our technique can be used to asses relative changes in platelet 5-HT transport induced by different antidepressants.

Dependence of platelet 5-HT transport on antidepressant concentration

After PRP from untreated rats ($n = 4$) was diluted, 0.2 ml of the sample (4×10^7 platelets) was mixed in plastic test tubes with KR solution and with 5 different antidepressant concentrations. The samples were preincubated at 37 ℃ for 60 min. The 5-HT uptake was started by addition of 0.5 ml [^{3}H]5-HT. The final sample volume was 4 ml (10^7 platelets/ml) and the final concentrations were: 1 μmol/l [^{3}H]5-HT; 0, 0.01, 0.1, 1, and 10 μmol/l of desipramine, maprotiline, citalopram, or moclobemide; 0, 0.002, 0.02, 0.2, and 2 mmol/l of lithium. The samples were incubated at 37 ℃ for 5 min, and reaction was terminated by rapid filtration through glass microfiber filters GF/C type (Whatman) soaked previously in 0.1 % polyethyleneimine (Sigma). Filters were washed rapidly 2 times with 3 ml of ice-cold saline. Filtration and washing were accomplished within 10 s. After adding the scintillation cocktail, the filters were assayed on LS 6000IC scintillation counter (Beckman Instruments Inc., Fullerton, CA, USA). The total uptake of 5-HT is assumed to be the sum of the high affinity transport (specific uptake) and the non-specific uptake, which was evaluated with 2 ℃ cold samples. Moreover, radiolabelled chemicals possess remarkable abilities to bind non-specifically to both biological and non-biological substances (Bennett and Yamamura 1985), e.g. to platelet surface or to filter. By subtracting the activity of filter that collected sample incubated at 2 ℃ from the activity of filter that captured the same sample incubated at 37 ℃, the high affinity transport was determined. The samples were measured in doublets.

Platelet 5-HT transport kinetics measurements

The technique employed in our study was method by analogy with receptor studies (Bennett and Yamamura 1985). After PRP from controls or antidepressant-treated rats was diluted, 0.2 ml of the sample (4×10^7 platelets) was mixed in plastic test tubes with KR solution and preincubated at 37 ℃ for 12 min. The measurements of platelet 5-HT uptake kinetics were initiated by adding 12 different [^{3}H]5-HT volumes of 8 μmol/l [^{3}H]5-HT so that the resultant concentrations of [^{3}H]5-HT for platelet uptake were in the range of 10 nmol/l and 1 μmol/l. The final volume of the testing sample was 4 ml with platelet concentration of 10^7 *per* ml. After adding labelled 5-HT the test tubes were filled with 95 % O_2 and 5 % CO_2 gas mixture, sealed and incubated at 37 ℃ for 5 min. Free [^{3}H]5-HT was then removed by rapid filtration as described above. The non-specific uptake and the non-specific binding of [^{3}H]5-HT to platelet surface or to filter were determined from 2 ℃ cold samples (see above). The samples at 37 ℃ were measured in doublets; the samples at 2 ℃ were measured in singlets.

Data analysis

Inhibition of platelet 5-HT uptake by antidepressants was analysed using the four-parametric logistic model (Rodbard et al. 1976; ImmunoFit EIA/RIA software, Beckman) to establish the values of drug concentration inhibiting 50 % of 5-HT uptake (IC_{50}). To calculate the parameters of kinetics of 5-HT platelet transport (V_{max} and K_M), we used AccuFit Saturation Two-Site nonlinear regression analysis software (Beckman). Limiting permeability at low (physiological) 5-HT concentrations was calculated as V_{max}/K_M ratio (Franke et al. 2000). V_{max}/K_M corresponds to second-order rate constant for transport mechanism multiplied by SERT molar concentration. Although we do not know SERT concentration, V_{max}/K_M can be considered as efficiency criterion of transport system (apparent 5-HT uptake efficiency).

Data are expressed as the arithmetic means. Standard deviation (SD) was calculated to characterize group variability. Hypothesis testing was performed using analysis of variance (ANOVA), followed by post hoc Duncan test. Statistical analyses were performed with the statistical package Statistica (StatSoft, Inc.).

Results

Different acute *in vitro* effects of antidepressants on the 5-HT uptake are demonstrated on Figure 1. As was to be expected, the platelet *in vitro* treatment with different antidepressant concentrations resulted in decrease of the 5-HT uptake for citalopram ($IC_{50} = 0.015 \pm 0.002$ μmol/l) > desipramine ($IC_{50} = 0.191 \pm 0.007$ μmol/l) > maprotiline ($IC_{50} = 3.48 \pm 0.06$ μmol/l), and no significant decrease was observed for moclobemide and lithium.

The assessment of changes in the activity of platelet SERT following long-term administration of 5 pharmacologically selective antidepressants was based on

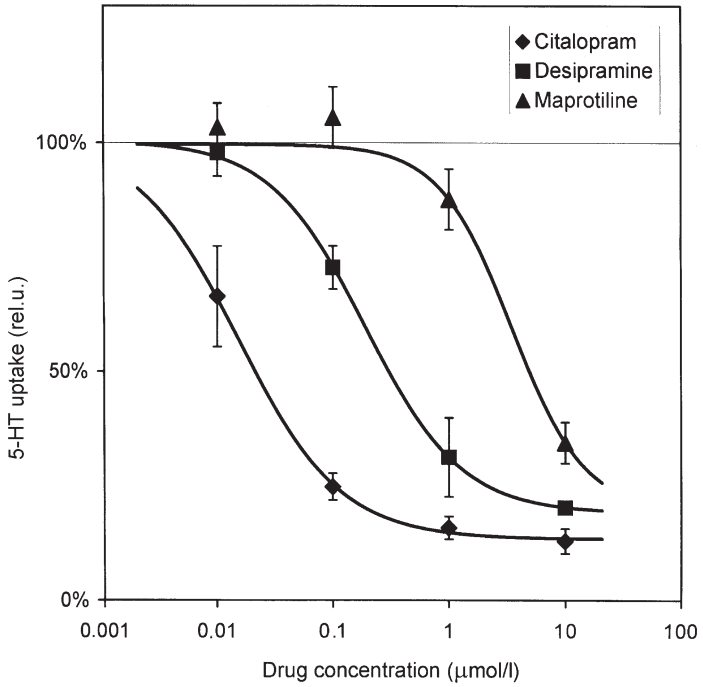

Figure 1. Dependence of 5-HT uptake on antidepressant concentration. Platelets from untreated rats were used; the 5-HT uptake was related to the value in absence of the drug. The final sample volume was 4 ml with platelet concentration of 10^7 *per* ml. The samples were incubated with antidepressants at 37 °C for 60 min and the uptake was started by the addition of 1 μmol/l [^{3}H]5-HT; following incubation at 37 °C for 5 min, the rapid filtration was used to separate free radioligand. The samples were measured in doublets and non-specific binding was deducted. The figure shows mean values of four repetitions of the experiment ± SD. The 4-parameter logistic model was used for fitting data and calculating results; the IC_{50} of 5-HT uptake was achieved at 0.015 μmol/l of citalopram, 0.191 μmol/l of desipramine or 3.48 μmol/l of maprotiline.

measurements of kinetic parameters of 5-HT uptake in rat platelets *ex vivo*. The adaptive changes in transmembrane 5-HT transport were studied. Eventual direct inhibitory effect of antidepressants on SERT function *ex vivo* was eliminated by the decrease in free drug concentration (see Discussion) which was achieved both by 24-h wash-out period before blood collection and by dilution of PRP before 5-HT uptake measurement (the final dilution of PRP by KR solution was about eighty times); remaining concentrations of antidepressants (including citalopram) were too low to affect platelet SERT activity. The results are summarized in Table 1. A significant decrease in K_M compared to control group values was discovered following maprotiline ($p = 0.024$), citalopram ($p = 0.0098$), moclobemide ($p = 0.026$) and lithium ($p = 0.0061$) administration; administration of desipramine had

Table 1. Platelet 5-HT uptake in laboratory rats following 4-week administration of various antidepressants

Group	Dose (mg/kg *per* day)	Primary biochemical effect	K_M (nmol/l)	V_{max} (pmol/min·10^7 platelets)	V_{max}/K_M (ml/min·10^7 platelets)
Controls	0		68.0 ± 28.0	6.6 ± 3.5	0.110 ± 0.060
Desipramine	10	NE reuptake inhibitor	62.9 ± 14.0	10.3 ± 4.5	0.176 ± 0.094
Maprotiline	10	selective NE reuptake inhibitor	40.8 ± 5.7*	9.4 ± 3.2	0.227 ± 0.059**
Citalopram	5	selective 5-HT reuptake inhibitor	36.1 ± 7.6**	9.0 ± 1.6	0.264 ± 0.082***
Moclobemide	25	reversible MAO-A inhibitor	42.0 ± 6.0*	5.4 ± 1.2	0.131 ± 0.031
$LiCO_3$	50	inositol monophosphatase inhibitor and other effects	33.6 ± 4.5**	6.2 ± 1.1	0.187 ± 0.031

Values are means ± SD; the means were calculated from 19 values in case of control group and from 6 values in all other groups. NE, norepinephrine; 5-HT, 5-hydroxytryptamine, serotonin; MAO-A, monoamine oxidase type A; V_{max}, maximal velocity of platelet 5-HT uptake; K_M, Michaelis constant; V_{max}/K_M, ratio representing limiting permeability at low (physiological) extracellular concentrations of 5-HT. Values different from those of control group are marked in case the difference is statistically significant: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; determined by ANOVA and post hoc Duncan test.

no significant influence on K_M values. There was certain increase in V_{max} values following the administration of desipramine ($p = 0.058$), maprotiline ($p = 0.14$), and citalopram ($p = 0.17$); however, the all V_{max} changes were not significant.

V_{max}/K_M ratio representing limiting permeability at low (i.e. physiological) extracellular concentrations of 5-HT was calculated (Table 1). Although there was systematic increase in the V_{max}/K_M ratio in all the tested drugs, significant changes were occurred only in maprotiline- ($p = 0.0054$) and citalopram- ($p = 0.00035$) treated rats.

Discussion

Our study is a contribution to the understanding of SERT role in the treatment of depressive disorder. We measured both acute and long-term effects of pharmacologically selective antidepressants on the platelet 5-HT uptake. Experimental animals were used because capacity of a drug to alter monoamine uptake *in vitro* does not

predict its potential to modulate monoamine transporters after administration *in vivo* (Fleckenstein et al. 1999). Interpretation of our results is aggravated by the facts that both the biochemical response of the rat after long-term administration of antidepressants need not be equal to the response in man and the action of antidepressants on disturbed neurotransmitter systems during depression can be rather different from their action in normal physiological conditions. There are also dissimilarities between platelets and neurons with respect to the turnover of 5-HT. Nevertheless, we relied on the previous findings that platelets can be used as a peripheral model of central serotonergic function.

Changes in platelet 5-HT transport produced by administration of antidepressants were determined by measurements of [^{3}H]5-HT uptake. Relative decrease in the amounts of [^{3}H]5-HT entrapped into platelets was determined after *in vitro* addition of drugs to platelets from untreated rats (Figure 1). Adaptive changes in platelet 5-HT transport following long-term administration of antidepressants with different pharmacological selectivity were determined in *ex vivo* experiment, when platelet [^{3}H]5-HT uptake was measured at different [^{3}H]5-HT concentrations and parameters K_M and V_{max} that characterize transport properties of the SERT were calculated (Table 1). It is known, that the kinetic of 5-HT transport in platelets is determined mostly by the properties of the SERT (Stahl and Meltzer 1978). The density of SERT molecules in the cell membrane is not the only factor which determines the V_{max}; e.g. storage granules which accumulate 5-HT very rapidly (Costa et al. 1977) may be of importance for the overall 5-HT uptake. Moreover, the capability to release the 5-HT after the transport has been completed was not taken into consideration in our experiments. Therefore, the calculated K_M and V_{max} reflect the net transport in intact platelets rather than true SERT activity.

Both density of SERT molecules in the plasma membrane and the endogenous level of 5-HT in platelets could affect 5-HT transport. No significant relationship between V_{max} and platelet 5-HT content was found in previous study (Franke et al. 2000); however, the existence of a functional relationship between platelet 5-HT content and apparent 5-HT uptake efficiency was established. An observed changes in the values of V_{max} constant (Table 1) corresponded with antidepressant-induced changes of the 5-HT platelet content as mentioned in the Introduction; i.e. desipramine, maprotiline and citalopram decreased platelet 5-HT concentrations (Alvarez et al. 1999b; Javors et al. 2000; Ashina et al. 2004) and increased V_{max}, moclobemide increased platelet 5-HT concentrations (Lestra et al. 1998) and decreased V_{max}, and lithium had no effect both on platelet 5-HT concentrations (Glue et al. 1986; Poirier et al. 1988; Artigas et al. 1989) and V_{max}. It seems that the variation in V_{max} induced by long-term administration of pharmacologically selective antidepressants could be accounted both for density of 5-HT uptake sites (Maguire et al. 1993) and for platelet 5-HT levels. It can be hypothesised that both SSRI and non-SSRI antidepressants may exhibit their effect on 5-HT transport by increase in limiting permeability of membranes for 5-HT, which could be evoked by lowering of the 5-HT concentration gradient across the platelet membrane. This conclusion is rather speculative, because changes in V_{max} observed in our study

were not statistically significant and we relied on previously published data about the effect of antidepressants on platelet 5-HT concentrations.

The activity of SERT also depends on the modulation of substrate affinity by several endogenous factors. A registered decrease in the values of K_M constant (Table 1) can be interpreted as an increase in the SERT affinity, which leads to more effective clearance of extracellular 5-HT, resulting in its increased intrasynaptic availability and decreased extracellular 5-HT concentration, if serotonergic synapse is inactive and SERT is not inhibited. Considering variable receptor affinity hypothesis (Shinitzki 1984; Bevan et al. 1989), we suppose that effect of antidepressants on lipid-protein interactions (Seydel et al. 1992) could be responsible for changes in K_M. The finding of significantly reduced apparent K_M is in opposition to the general view that K_M constant is not changed during pharmacotherapy of depression.

Our results show that in spite of the 5-HT uptake inhibition observed in the acute *in vitro* experiments with citalopram, desipramine, and maprotiline (Figure 1), enhancement of the SERT activity is indicated by the observed changes in V_{max}/K_M ratio following the 4-week premedication with these drugs (Table 1). This can be accounted for the fact that adaptive changes in 5-HT transport were measured, which can be opposite to the acute effect of antidepressants on SERT activity. The most marked difference between *in vitro* and *ex vivo* results was found for citalopram.

Citalopram is the most selective and the most potent inhibitor of 5-HT reuptake. SSRI increase brain extracellular 5-HT concentrations in animals acutely following administration (Fuller 1994). However, the clinical effects are delayed for several weeks (Baumann 1992). This notion suggests the occurrence of adaptive changes triggered by the sustained elevated 5-HT levels. These changes involve desensitization of inhibitory 5-HT autoreceptors. The final increase in the overall serotonergic neurotransmission has been hypothesized to underlie the therapeutic effects of SSRIs (Blier and de Montigny 1994). The clinically effective plasma levels of citalopram in humans range between 0.12–0.84 μmol/l (Baumann 1992). In rats, similar plasma levels of citalopram were obtained at doses used in the present study (Cremers et al. 2000a). Elimination of drugs is, in general, much faster in rodents than in humans. Due to rapid drug elimination in rats, citalopram plasma levels dropped below 1/7 of stable citalopram level after 24 wash-out period (Cremers et al. 2000b) and extracellular levels of 5-HT in control and citalopram-treated rats were similar (Moret and Briley 1996). In our *ex vivo* uptake experiments, we used 24 wash-out period and PRP was diluted about 80 times before measurement; final free citalopram concentrations dropped below the threshold of the pharmacologically active plasma concentrations (Cremers et al. 2000b). Therefore, we really measured adaptive changes in platelet 5-HT transport evoked by long-term citalopram administration and direct effect of citalopram remaining in highly diluted PRP samples can be ruled out. The same conclusion is valid also for desipramine, maprotiline, moclobemide, and lithium-treated rats, because direct inhibitory effects of these drugs on SERT activity are much less when compared with citalopram.

Baseline levels of 5-HT in dialysate samples obtained from different brain areas varied between 0.1 and 1.0 nmol/l (Bel and Artigas 1999; Eriksson et al. 1999; Hjorth and Auerbach 1999) and uptake inhibitors cause about a 3- to 5-fold increase in extracellular 5-HT (Fuller 1994) so SERT is unsaturated at physiological conditions ($K_M > 30$ nmol/l), except for SERT in synaptic membrane immediately after 5-HT release. V_{max}/K_M ratio representing limiting permeability at low extracellular concentrations of 5-HT may be a proper parameter describing relative changes in total SERT activity. We found an increase in this ratio after a long-term administration of all antidepressants used including lithium. Citalopram and maprotiline induced the significant increase in V_{max}/K_M ratio while moclobemide showed the least effect (Table 1), which reflect potent effect of long-term administration of both selective 5-HT and selective NE reuptake inhibitors on the platelet 5-HT transport. The SERT is associated not only with synaptic membranes but also with axons (Zhou et al. 1998) so the increase in V_{max}/K_M may also affect the extrasynaptic 5-HT transmission. Because desipramine (potent NE reuptake inhibitor), citalopram (SSRI antidepressant), maprotiline (selective NE reuptake inhibitor antidepressant), moclobemide (reversible monoamine oxidase type A inhibitor antidepressant) and lithium (mood stabilizer with various effects on signal transduction) had qualitatively the same effect on V_{max}/K_M values, it can be assumed that this might be integral part of the adaptive changes in platelet 5-HT transport following long-term administration of pharmacologically selective antidepressants. Whether this is significant in the mechanism of therapeutic action of non-SSRI antidepressants it remains to be established.

Our results support 5-HT hypothesis of affective disorders, which is based on the assumption that insufficient 5-HT activity is an important factor of vulnerability to depression and that one of the manifestation of long-term antidepressant therapy is the increase in brain serotonergic activity (Meltzer and Lowy 1987; Caldecott-Hazard et al. 1991; Siever et al. 1991; Charney and Delgado 1992; Stahl 1992, 1994; Maes and Meltzer 1995). This antidepressant-induced increase in 5-HT activity is probably based both on desensitisations of inhibitory 5-HT autoreceptors and on adaptive changes in transmembrane 5-HT transport. Changes in SERT affinity and transport velocity cannot fully manifest during the administration of SSRI, because the transport is largely blocked and 5-HT concentration is increased in extracellular space and reduced in cytoplasm. When final free SSRI concentrations dropped below the threshold of the pharmacologically active plasma concentrations (after a sufficient wash-out period or dilution), the combination of increased SERT affinity and unchanged or increased transport velocity results in more complete 5-HT clearance from the extracellular space and in increased possibility of its reuse. From this point of view, the increases in extracellular 5-HT concentrations during administration of SSRI could participate in induction of their therapeutic effects, and increase in 5-HT transport after drug withdrawal could participate in the maintenance of effects of pharmacotherapy. The increase in limiting permeability of the membrane for 5-HT could be included in the common adaptive effect of the long-term administration of different antidepressants (both SSRI and

non-SSRI). If such 5-HT uptake changes occur in the brain, therapeutic effects of desipramine, maprotiline, moclobemide or lithium might be partially due to action on SERT function.

Acknowledgements. This work was supported by the GA UK 27/2000/C and MSM 111100001 grants.

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Final version accepted: January 25, 2005