

Inhibition of monoamine oxidase activity by antidepressants and mood stabilizers

Zdeněk FIŠAR, Jana HROUDOVÁ, Jiří RABOCH

Department of Psychiatry, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Prague, Czech Republic

Correspondence to: Assoc. Prof. Zdeněk Fišar, CSc.
Department of Psychiatry, First Faculty of Medicine,
Charles University in Prague and General University Hospital in Prague,
Ke Karlovu 11, 120 00 Prague 2, Czech Republic.
TEL/FAX: +420224965313; E-MAIL: zfishar@lf1.cuni.cz

Submitted: 2010-01-14 Accepted: 2010-04-02 Published online: 2010-12-05

Key words: antidepressive agents; monoamine oxidase inhibitors; mood stabilizers

Neuroendocrinol Lett 2010; 31(5):645–656 PMID: 21200377 NEL310510A19 © 2010 Neuroendocrinology Letters • www.nel.edu

Abstract

OBJECTIVE: Monoamine oxidase (MAO), the enzyme responsible for metabolism of monoamine neurotransmitters, has an important role in the brain development and function, and MAO inhibitors have a range of potential therapeutic uses. We investigated systematically *in vitro* effects of pharmacologically different antidepressants and mood stabilizers on MAO activity.

METHODS: Effects of drugs on the activity of MAO were measured in crude mitochondrial fraction isolated from cortex of pig brain, when radiolabeled serotonin (for MAO-A) or phenylethylamine (for MAO-B) was used as substrate. The several antidepressants and mood stabilizers were compared with effects of well known MAO inhibitors such as moclobemide, iproniazid, pargyline, and clorgyline.

RESULTS: In general, the effect of tested drugs was found to be inhibitory. The half maximal inhibitory concentration, parameters of enzyme kinetic, and mechanism of inhibition were determined. MAO-A was inhibited by the following drugs: pargyline > clorgyline > iproniazid > fluoxetine > desipramine > amitriptyline > imipramine > citalopram > venlafaxine > reboxetine > olanzapine > mirtazapine > tianeptine > moclobemide, cocaine >> lithium, valproate. MAO-B was inhibited by the following drugs: pargyline > clorgyline > iproniazid > fluoxetine > venlafaxine > amitriptyline > olanzapine > citalopram > desipramine > reboxetine > imipramine > tianeptine > mirtazapine, cocaine >> moclobemide, lithium, valproate. The mechanism of inhibition of MAOs by several antidepressants was found various.

CONCLUSIONS: It was concluded that MAO activity is acutely affected by pharmacologically different antidepressants at relatively high drug concentrations; this effect is inhibitory. There are differences both in inhibitory potency and in mechanism of inhibition between both several drugs and the two MAO isoforms. While MAO inhibition is not primary biochemical effect related to their therapeutic action, it can be supposed that decrease of MAO activity may be concerned in some effects of these drugs on serotonergic, noradrenergic, and dopaminergic neurotransmission.

Abbreviations:

AICc	- Akaike's information criterion
DALY	- disability adjusted life years
FAD	- flavin adenine dinucleotide
H ₂ O ₂	- hydrogen peroxide
5-HT	- serotonin, 5-hydroxytryptamine
IC ₅₀	- half maximal inhibitory concentration
K _i	- dissociation constant of the enzyme-inhibitor complex
K _m	- Michaelis constant
MAO	- monoamine oxidase
MAOI	- monoamine oxidase inhibitor
NaSSA	- noradrenergic and specific serotonergic antidepressant
NDRI	- norepinephrine-dopamine reuptake inhibitor
NE	- norepinephrine
NRI	- norepinephrine reuptake inhibitor
PEA	- 2-phenylethylamine
RIMA	- selective reversible MAO-A inhibitor
S	- substrate concentration
SNRI	- serotonin-norepinephrine reuptake inhibitor
SSRE	- selective serotonin reuptake enhancer
SSRI	- selective serotonin reuptake inhibitor
TCA	- tricyclic antidepressant
V _{max}	- maximum rate

INTRODUCTION

Depression is a serious mental disorder that manifests with depressed mood, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, low energy, and poor concentration. The estimated lifetime prevalence of having mood disorders varies from 3.3 to 21.4% in several countries (Kessler *et al.* 2007). The World Health Organization currently ranks depression as the leading cause of disability as measured by YLDs (Years Lived with Disability) and the 4th leading contributor to the global burden of disease (Disability Adjusted Life Years, DALYs) in 2000. By the year 2020, depression is projected to reach 2nd place of the ranking of DALYs (World Health Organization Web site. Depression. http://www.who.int/mental_health/management/depression/definition/en/).

Current antidepressant medications are effective in about 60% of treated patients (Nelson 1999; Papakostas *et al.* 2007). All clinically effective antidepressants affect serotonergic and/or noradrenergic signal transduction pathways (Fišar & Hroudová 2010). Antidepressant medications targeting the monoamine systems have been available since the late 1950s. The observation that iproniazid, a drug that effectively blocked monoamine oxidase (MAO) activity, or imipramine, a tricyclic compound that acted by inhibiting norepinephrine (NE) and serotonin (5-HT, 5-hydroxytryptamine) reuptake, had antidepressant effects led to the discovery of other compounds that modulate the 5-HT and NE neurotransmitter systems and could be used as effective antidepressants.

Discovery of the first effective antidepressants, monoamine oxidase inhibitors (MAOIs) and tricyclic antidepressants (TCAs), implied hypothesis about significant role for the biogenic amine, particularly NE

and 5-HT in the etiopathogenesis of affective disorders. Monoamine hypothesis is an early milestone in the field of depression (Hindmarch 2002).

The monoamine hypothesis postulates that depression is due to a deficiency of monoamine neurotransmitters, NE and 5-HT at key sites in the brain. MAOIs act as antidepressants by blocking of enzyme that degrades monoamine neurotransmitters; TCAs act as antidepressants by blocking membrane transporters ensuring reuptake of 5-HT or NE, thus causing increased extracellular neurotransmitter concentrations. But the monoamine hypothesis of depression in itself could neither explain the whole mechanism of action of antidepressants, nor could it provide an explanation for how monoamine loss occurs. According to advanced monoamine theory (Meyer *et al.* 2006), serotonin or norepinephrine levels in the brain are regulated by monoamine oxidase type A (MAO-A) activity mainly. During a major depressive episode, MAO-A density is elevated, resulting in greater metabolism of monoamines in the brain. However, severity of symptoms of depression is related to changes in the activity of monoamine transporters in specific brain regions. So, both MAO-A activity and density of transporters are included in the pathophysiology of affective disorders.

The antidepressant activity of the TCAs and MAOIs has supported the role of NE and 5-HT in depression and the mechanism involved in antidepressant action. The next generation of antidepressants included selective serotonin reuptake inhibitors (SSRIs), norepinephrine reuptake inhibitors (NRIs), serotonin-norepinephrine reuptake inhibitors (SNRI), noradrenergic and specific serotonergic antidepressants (NaSSAs), norepinephrine-dopamine reuptake inhibitors (NDRIs), selective serotonin reuptake enhancer (SSRE), melatonergic agonists etc.

Mood stabilizers are psychiatric medication used in treatment of mood disorders characterized by intense and sustained mood shifts (e.g. bipolar affective disorder). Most mood stabilizers are anticonvulsants, with the important exception of lithium, which is the oldest and the best known mood stabilizing drug. Some atypical antipsychotics have also mood stabilizing effects.

The enzyme monoamine oxidase (MAO; EC1.4.3.4) is a mitochondrial enzyme, which catalyzes oxidative deamination of biogenic and xenobiotic monoamines. It regulates the metabolic degradation of catecholamines and 5-HT in neural and other target tissues. Major physiological role of intraneuronal MAO is to keep cytosolic monoamine concentrations very low. MAO exists in two isoforms that differ in substrate preference, inhibitor specificity, tissue and cell distribution, and immunological properties. Sequences for these forms of MAO were appeared in 1988 (Bach *et al.* 1988). The type A (MAO-A) metabolizes 5-HT and is sensitive to inhibition by low concentrations of clorgyline, whereas the type B (MAO-B) prefers benzylamine or 2-phenylethylamine (PEA) as substrate and is sensi-

tive to inhibition by low concentrations of l-deprenyl. Tyramine, tryptamine, dopamine, norepinephrine and epinephrine are equally well oxidized by both isoforms of MAO (Youdim *et al.* 2006). The high levels of both forms are found in the brain; MAO-B is found in dopamine-secreting neurons in the brain.

MAO is located on the outer mitochondrial membrane and is a flavoprotein that contains the flavin adenine dinucleotide (FAD) as cofactor. This cofactor was identified as the site at which irreversible inhibitors of MAO are covalently linked (Youdim & Bakhle 2006; Youdim *et al.* 2006). Small proportion of MAO is associated with the microsomal fraction (Gómez *et al.* 1988). There are three functionally distinct domains in MAO molecule: substrate domain, flavin-binding domain, and region that attaches the protein to the mitochondrial membrane (Fowler *et al.* 2007; Edmondson *et al.* 2009). Human MAO-A is a monomer, MAO-B is a dimer.

Monoamine metabolism by MAO involves oxidative deamination to corresponding aldehyde and free amine. Catalysis in MAO depends on the transfer of electrons to FAD, and mechanism-based inhibitors, such as the irreversible antidepressants, modify flavin (Ramsay & Gravestock 2003). The aldehyde is rapidly metabolized by aldehyde dehydrogenase to acidic metabolites. Metabolism of monoamines by MAO is the major source of hydrogen peroxide (H₂O₂) in the brain. Normally the H₂O₂ is then inactivated by glutathione peroxidase but it can be converted, chemically, by Fe²⁺ ions (Fenton reaction) into the highly reactive hydroxyl radical. This radical has widespread deleterious effects, which can cause neuronal damage and death and may account for associated health-related problems (Youdim & Bakhle 2006).

MAOs have an important role in brain development and function, and MAOIs have a range of potential therapeutic uses (Ramsay & Gravestock 2003). Generally, selective inhibitors of MAO-A and nonselective MAOIs seem to be effective in the treatment of patients with depression, panic disorder, and other anxiety disorders (Stahl & Felker 2008). It is supposed that MAO-B inhibition may slow the course of various neurodegenerative disorders; so, selective inhibitors of MAO-B may be efficacious in treating of Parkinson's disease (Horstink *et al.* 2006) and possibly Alzheimer's disease (Riederer *et al.* 2004). MAO-B is the sole type in human platelets and the amino acid sequences of MAO-B in both platelets and brain are identical (Chen *et al.* 1993); so, platelet MAO can be adopted as a useful surrogate model for the study of aspects of central neuronal function related to monoaminergic neurotransmission (Fišar & Raboch 2008).

The antidepressant effects of MAO-A inhibition with non-selective inhibitors was already established. Iproniazid became the first MAO inhibitor that was used successfully in the treatment of depression; it is an irreversible and nonselective MAO inhibitor (Fagervall

& Ross 1986). It is known as a pro-drug and can be converted into isopropyl hydrazine, which binds covalently to MAO (Smith *et al.* 1963). Clorgyline is an irreversible inhibitor preferential for MAO-A, structurally related to pargyline (MAO-B inhibitor). It has antidepressant activity, and may be potentially useful in the treatment of Parkinson's disease. Selegiline (l-deprenyl) is an irreversible inhibitor preferential for MAO-B; it is used for the treatment of Parkinson's disease, depression and senile dementia. Inhibitors of MAO lose their selectivity at high doses. Moreover, there are feedbacks and interconnections of intracellular signalling pathways that lead to mutual interactions of monoaminergic systems (Fišar & Hroudová 2010). Therefore, inhibiting of MAO-B should influence processes mediated primarily by substrates for MAO-A, and vice versa. The main disadvantage was the incidence of the cheese reaction with those early inhibitors (Youdim & Bakhle 2006).

The selective reversible MAO-A inhibitors (RIMA), such as moclobemide, increase the quantity of serotonin, norepinephrine and dopamine in the brain (Haeffely *et al.* 1992) but do not provoke the cheese reaction. Moclobemide has been extensively evaluated in the treatment of a wide spectrum of depressive disorders and social phobia. Generally, moclobemide appears to be safe and devoid of major side effects, although it is considered as a mild antidepressant, better tolerated by older patients (Lecrubier 1990, 1994; Iwersen & Schmoldt 1996; Lotufo-Neto *et al.* 1999; Madger *et al.* 2000; Bonnet 2003; Praško *et al.* 2006). Moclobemide undergoes extensive metabolism with less than 1% of the dose being excreted unchanged. Metabolic pathways of moclobemide include mainly oxidative attack on the morpholine moiety (Jauch *et al.* 1990). However, major metabolites in plasma were found to be less effective MAO-A inhibitors than moclobemide or pharmacologically inactive (Da Prada *et al.* 1989; Baker *et al.* 1999).

MAO inhibitors were developed as antidepressants but many drugs, including the oxazolidinone antibacterial agents, share similar molecular properties and have MAO inhibitory activity. These compounds were of interest as potential antidepressants because they could be selective inhibitors of either A or B isoforms and were usually reversible (Ramsay & Gravestock 2003).

Antidepressants, which act primarily as serotonin and/or norepinephrine reuptake inhibitors, show inhibitory activity towards MAO also. It has been suggested that tricyclic antidepressants exert some of their therapeutic effect by inhibiting MAO (Reyes & Lisanisky 1984). It was described that they are able to inhibit MAO-B both *in vitro* (Roth & Gillis 1974; Edwards & Burns 1974) and *in vivo* (Sullivan *et al.* 1977, 1978). However, *in vivo* inhibition of the human platelet MAO-B in patients taking tricyclic antidepressants was not confirmed by others (Davidson *et al.* 1978; Reveley *et al.* 1979). Five TCAs, amitriptyline, clomipramine, desipramine, imipramine and iprindole, have compa-

rable potencies as inhibitors of monoamine oxidase in rodent brain and liver (Green & McGachy 1987). These antidepressants have been shown to partially protect mouse brain monoamine oxidase *in vivo* from the irreversible enzyme inhibition produced by subsequent injection of phenelzine (Green *et al.* 1989). TCAs concentrations, which showed a pronounced inhibitory effect on the MAOs activity, were significantly higher than plasma levels of the drug found under therapeutic conditions (Honecker *et al.* 1976; Nag 2004). MAOs activity was inhibited after long-term administration of viloxazine, nomifensine, zimelidine, maprotiline, imipramine, amitriptyline, and nortriptyline in systematic studies of Egashira *et al.* (1996, 1999). Competitive inhibition of MAO-A and non-competitive inhibition of MAO-B was found for these drugs. Similar results were obtained when different TCAs and SSRIs were examined with isolated rat brain mitochondria (Gnerre *et al.* 2001). Fluoxetine and norfluoxetine showed affinities both for MAO-A (Mukherjee & Yang 1999) and MAO-B (Mukherjee & Yang 1997). Fluoxetine and norfluoxetine also significantly inhibited the binding of the specific radioligands to MAOs *in vivo*. These results support a potential role of MAO inhibition in the therapeutic effects of fluoxetine.

Effect of mood stabilizers (carbamazepine, divalproex, lamotrigine, lithium, or an atypical antipsychotic) on monoaminergic activity was studied too (Redrobe & Bourin 1999; Chen & Chen 2007). There are most data about effects of lithium. Lithium enhances the antidepressant effect both of MAOIs and inhibitors of the reuptake of serotonin and/or norepinephrine (Magder *et al.* 2000; Nierenberg *et al.* 2006; Kitaichi *et al.* 2006). The mode of action for the lithium augmentation of antidepressants is partly mediated by an increase of 5-HT neurotransmission (Redrobe & Bourin 1999; Haddjeri *et al.* 2000; Muraki *et al.* 2001). However, lithium could neither inhibit MAO-A or MAO-B in the mitochondrial fraction of rat brain (Nag 2004). Unipolar and bipolar depressive patients showed significantly higher platelet MAO activity than controls, but there was no significant change in activity after the institution of lithium treatment (Reveley *et al.* 1981).

Incomplete data exist on the effect of pharmacologically different antidepressants and mood stabilizers on MAO activity. The present study was designed to analyze effects of pharmacologically different antidepressants (desipramine, amitriptyline, imipramine, fluoxetine, citalopram, venlafaxine, reboxetine, mirtazapine, tianeptine, moclobemide), and mood stabilizers (lithium, valproate, olanzapine) on MAO activity. The *in vitro* effects of these drugs on MAO activities in mitochondria isolated from pig brains were examined. Brain mitochondria were assayed for MAO-A and MAO-B using 5-HT and PEA as substrates. One way to determine an inhibitory effect of a drug is to measure enzyme rate at a single concentration of substrate with varying drug concentrations to determine the disso-

ciation constant of the enzyme-inhibitor complex (K_i). Another experimental design is to measure enzyme rate at a variety of substrate concentrations in the presence and absence of an inhibitor to determine the mechanism of inhibition.

MATERIAL & METHODS

Pig brain mitochondria isolation

Fresh pig brains were obtained from slaughter-house and immediately placed to ice-cold buffered sucrose (0.32 mol/l sucrose, 4 mmol/l HEPES; pH 7.4). All subsequent procedures were performed at 0–4 °C. Brain cortex was separated without cerebellum, gently homogenized in ten volumes (w/v) of ice-cold 0.32 mol/l buffered sucrose supplemented with aprotinin (competitive serine protease inhibitor), by means of a homogenizer with Teflon piston. Mitochondria were prepared by a standard differential centrifugation method (Whittaker 1969). Briefly, the homogenate was centrifuged at 1 000 g for 10 min to remove unbroken cells, nuclei and cell debris. The supernatant was carefully decanted; the pellet was resuspended in buffered sucrose and centrifuged again under the same conditions. Supernatants were collected and recentrifuged at 10 000 g for 15 min. The final pellet containing mitochondria was rinsed twice with buffered sucrose (10 000 g, 15 min), resuspended to a protein concentration of 20–40 mg/ml, and stored at –70 °C until the assays. Protein concentration was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as the standard.

Measurement of MAO activity

MAOs activity was determined radiochemically (Ekstedt 1976; Egashira *et al.* 1978; Ozaita *et al.* 1997; Egashira *et al.* 1999) with either [¹⁴C]5-HT (maximum concentration 100 μmol/l) or [¹⁴C]PEA (maximum concentration 10 μmol/l) as substrates. At such concentrations these amines have been shown to behave as specific substrates for A and B isoforms of MAO, respectively (Fowler & Tipton 1981; Youdim *et al.* 2006).

Mitochondria were diluted in modified Krebs-Henseleit buffer without Ca²⁺ (KH solution; 118 mmol/l NaCl, 4.7 mmol/l KCl, 1.2 mmol/l KH₂PO₄, 1.2 mmol/l MgSO₄, 25 mmol/l NaHCO₃, 11.1 mmol/l glucose; pH 7.4). The reaction mixture containing the crude MAO and various drug concentrations was preincubated for 60 min at 37 °C. Reaction was started by addition of radiolabeled substrate and MAO activity was measured at 37 °C for 30 min for MAO-A and for 1 min for MAO-B.

MAO-A activity was measured using [¹⁴C]5-HT solution with specific activity of approximately 40 kBq/ml, which was prepared by mixing of [¹⁴C]5-HT stock solution (5-hydroxytryptamine binoxalate 5-[2-¹⁴C]), specific activity of 1.85 GBq/mmol, radioactive concentration 3.7 MBq/ml, radiochemical purity greater than 99%, American Radiolabeled Chemicals, Inc., St. Louis,

MO, USA) with KH buffer and unlabeled (cold) serotonin (5-hydroxytryptamine hydrochloride, Sigma-Aldrich Co., St. Louis, MO, USA).

MAO-B activity was measured using [^{14}C]PEA solution with specific activity of approximately 40 kBq/ml, which was prepared by mixing of [^{14}C]PEA stock solution (2-phenylethylamine [ethyl-1- ^{14}C] hydrochloride, specific activity of 2.03 GBq/mmol, radioactive concentration 3.7 MBq/ml, radiochemical purity greater than 99%; American Radiolabeled Chemicals) with unlabeled PEA (2-phenylethylamine hydrochloride; Sigma-Aldrich Co.) solution in KH buffer.

The final sample volume was 250 μl containing 200 μg of protein. The reaction was stopped by the addition of 250 μl of 2 N hydrochloric acid. The reaction products, i.e. the corresponding aldehydes of 5-HT and PEA after oxidative deamination, were extracted into benzene:ethyl acetate 1:1 (v/v) and the radioactivity of the extracts in the organic phase were measured by liquid scintillation counting. Both MAO assays were performed over times where product formation was shown to proceed linearly under the condition used so that the obtained values corresponded to the initial velocities of the enzyme-catalyzed reaction.

Since some drugs are poorly soluble in aqueous media, they were sonicated in KH solution to obtain homogenous suspension. Iproniazid was used in the form of phosphate salt; clorgyline and pargyline were used in the form of hydrochlorides (all purchased from Sigma-Aldrich Co.).

Inhibition of MAO-A and MAO-B activities

Dependence of MAO activity on drug concentration was measured at different final concentrations (at least 7 concentrations). The effects of desipramine, amitriptyline, imipramine, fluoxetine, citalopram, venlafaxine, reboxetine, mirtazapine, tianeptine, moclobemide, lithium, valproate, olanzapine, iproniazid, pargyline, clorgyline, and cocaine were examined. Blank values were obtained by addition of the hydrochloric acid before the substrate was added. MAO-A activity was measured using 16 $\mu\text{mol/l}$ [^{14}C]5-HT solution. The final [^{14}C]5-HT concentration in samples was 3.2 $\mu\text{mol/l}$. MAO-B activity was measured using 50 $\mu\text{mol/l}$ [^{14}C]PEA solution. The final [^{14}C]PEA concentration in samples was 10 $\mu\text{mol/l}$. The remaining MAO-A and MAO-B activities were expressed as portions of control basal activity and plotted as a function of the drug concentration. The reversibility of the inhibition of MAO by drugs was proven using the dilution method (Ulus *et al.* 2000).

Determination of enzyme kinetic parameters

Steady-state kinetic constants (K_m , Michaelis constant and V_{max} , maximum rate) were determined from studies of the effects of substrate concentration on the initial reaction rate of MAO-A or MAO-B in the absence of drugs and in the presence of different concentrations of drugs.

Kinetic constants for MAO-A were assessed with seven different concentrations of [^{14}C]5-HT (5, 8, 10, 12.5, 25, 50, 100 $\mu\text{mol/l}$) using aliquots of 250 $\mu\text{mol/l}$ [^{14}C]5-HT solution. Blank values were obtained by the addition of 100 $\mu\text{mol/l}$ clorgyline before the substrate was added.

Similarly, kinetic constants for MAO-B were determined with seven different concentrations of [^{14}C]PEA (0.5, 0.8, 1, 1.25, 2.5, 5, 10 $\mu\text{mol/l}$) using aliquots of 25 $\mu\text{mol/l}$ [^{14}C]PEA solution. Blank values were obtained by the addition of 1 $\mu\text{mol/l}$ pargyline before the substrate was added.

Data analysis

Inhibition of MAO activity by drugs was analyzed using the four-parameter logistic function (SigmaPlot, Systat Software, Inc., Richmond, CA, USA), to establish the half maximal inhibitory concentration (IC_{50}) and Hill slope (coefficient). Hill slope characterizes the slope of the curve at its midpoint and it is used in determining the degree of cooperativity of the ligand binding to the enzyme. IC_{50} represents the concentration of a drug that is required for 50% inhibition of enzymic reaction at a specific substrate concentration. IC_{50} values are dependent on conditions under which they are measured; so, data obtained are valid for existing enzyme concentration and type of inhibition. Because inhibitory potency is related to the dissociation constant of the enzyme-inhibitor complex (K_i , the reciprocal of the binding affinity of the inhibitor to the enzyme) this parameter can be a predictor of *in vivo* inhibitory potency. The analysis shown that K_i is equal to IC_{50} under conditions of either non-competitive or uncompetitive kinetics; however, K_i does not equal IC_{50} when competitive inhibition kinetics applies (Cheng & Prusoff 1973). IC_{50} and K_i can be related to competitive reversible inhibitor of monosubstrate reaction by Cheng-Prusoff equation:

$$K_i = IC_{50} / (1 + S/K_m)$$

where S is substrate concentration.

The MAO activity obeyed simple Michaelis-Menten kinetics with maximum rate V_{max} and Michaelis constant K_m (numerically equal to the substrate concentration required at rate of $V_{\text{max}}/2$). Different effects of the inhibitor on V_{max} and K_m result from its binding to the enzyme, to the enzyme-substrate complex, or to both. Reversible enzyme inhibitors can be classified as competitive, non-competitive, uncompetitive, or mixed. We used nonlinear regression (SigmaPlot with Enzyme Kinetics Module) to calculate parameters V_{max} , K_m , and to determine the mechanism of inhibition. Akaike's information criterion (Akaike 1974) with a second order correction for small sample sizes (AICc) was used as a tool for model selection, i.e. as a measure of the goodness of fit of an estimated type of inhibition.

RESULTS

The effects of antidepressants (desipramine, amitriptyline, imipramine, fluoxetine, citalopram, venlafaxine, reboxetine, mirtazapine, tianeptine, moclobemide) and mood stabilizers (lithium, valproate, olanzapine) on MAOs activity and nature of the interaction in pig brain mitochondria were assessed in comparison with iproniazid, pargyline, clorgyline, and cocaine. As was to be expected, the mitochondria treatment with iproniazid, pargyline or clorgyline resulted in decrease of the MAOs activity at nanomolar to micromolar drug concentrations (Figures 1C, 2C). IC_{50} values were determined by analyzing dose-response curves of the MAO activity against the drug concentration. It must be noted that irreversible inhibitors (iproniazid, pargyline, clorgyline) display time-dependent inhibition and their potency cannot be correctly characterized by IC_{50} value.

Potency of tested drug in inhibiting MAO-A activity is summarized in the Table 1. Most of the drugs were relatively potent in inhibiting MAO-A activity, whereas MAO-A was not fully inhibited at both high mirtazapine and olanzapine concentrations (lower efficacy). The residual activities of MAO-A were about 21.9%, and 29.1% with mirtazapine and olanzapine, respectively (Figure 1). Amitriptyline, imipramine, fluoxetine, mir-

tazapine and tianeptine displayed positive cooperativity of the binding to MAO-A. Venlafaxine, moclobemide and olanzapine displayed negative cooperativity. A Hill coefficient of 1 indicates completely independent binding for desipramine, citalopram and reboxetine.

The effects of the same drugs were also evaluated on pig brain mitochondrial MAO-B activity using PEA as substrate (Table 2). Most of drugs were relatively potent in inhibiting MAO-B activity. Similarly to MAO-A, MAO-B was not fully inhibited at both high mirtazapine and olanzapine concentrations; however, lower efficacy of inhibition was found for venlafaxine, amitriptyline and reboxetine too. The residual activities of MAO-B were about 29.0%, 32.2%, 58.5%, 17.3% and 39.9% with venlafaxine, amitriptyline, olanzapine, reboxetine and mirtazapine, respectively (Figure 2). Fluoxetine, venlafaxine, amitriptyline and tianeptine displayed positive cooperativity of the binding to MAO-B; olanzapine displayed negative cooperativity.

Lithium and valproate are very weak inhibitors of both isoforms of MAO. Moclobemide does not inhibit MAO-B and it is weak *in vitro* inhibitor of MAO-A.

Isothermal saturation curves for MAOs were measured following incubation of mitochondria with different concentrations of radiolabeled substrates and kinetic parameters of MAOs activity were calculated

Tab. 1. Inhibition of MAO-A activity in brain crude mitochondrial fraction.

	IC_{50} $\mu\text{mol/l}$	Hill slope	Mechanism of inhibition	N
Fluoxetine	16.8 ± 1.0	1.38 ± 0.10	Non-competitive	5
Desipramine	29.2 ± 2.8	1.00 ± 0.09	Mixed	7
Amitriptyline	38.2 ± 2.9	1.31 ± 0.11	Mixed	8
Imipramine	50.5 ± 3.5	1.37 ± 0.10	Mixed	6
Citalopram	55.0 ± 5.4	1.03 ± 0.09	Non-competitive	8
Venlafaxine	83.5 ± 12.6	0.75 ± 0.08	Non-competitive	8
Reboxetine	197 ± 23	1.12 ± 0.13	Non-competitive	7
Olanzapine	212 ± 71	0.62 ± 0.10	Competitive	5
Mirtazapine	235 ± 30	1.33 ± 0.21	Non-competitive	9
Tianeptine	469 ± 39	1.65 ± 0.17	Uncompetitive	7
Moclobemide	1105 ± 101	0.77 ± 0.05	Competitive	10
Lithium	>10000			6
Valproate	>10000			6
Pargyline	0.01152 ± 0.00067	1.68 ± 0.18	Irreversible	12
Clorgyline	0.347 ± 0.041	1.12 ± 0.13	Irreversible	14
Iproniazid	1.55 ± 0.12	1.16 ± 0.10	Irreversible	16
Cocaine	1060 ± 105	0.993 ± 0.084	Mixed	8

The samples were incubated with drugs at 37 °C for 60 minutes and the reaction was started by addition of 3.2 $\mu\text{mol/l}$ [^{14}C]serotonin; following incubation at 37 °C for 30 minutes, the reaction was stopped by addition of hydrochloric acid. The samples were measured in doublets and blank values were deducted.

Values are means ± standard deviation. IC_{50} = drug concentration that is required for 50% inhibition of enzyme activity; Hill slope = describes the cooperativity of the drug binding; N = number of measurements.

using nonlinear regression analysis (SigmaPlot with Enzyme Kinetics Module). Kinetic parameters of MAO activity in pig brain crude mitochondrial fraction towards 5-HT as substrate demonstrated $V_{\max} = 24.8 \pm 5.3$ pmol/(min · mg protein), $K_m = 6.7 \pm 2.3$ μ mol/l, and $V_{\max}/K_m = 3.89 \pm 0.68 \cdot 10^{-6} \cdot$ l/(min · mg protein) (mean \pm SD, $N = 15$). Kinetic parameters of MAO activity towards PEA as substrate were calculated $V_{\max} = 474 \pm 79$ pmol/(min · mg protein), $K_m = 0.43 \pm 0.14$ μ mol/l, and $V_{\max}/K_m = 1182 \pm 276 \cdot 10^{-6} \cdot$ l/(min · mg protein) (mean \pm SD, $N = 14$).

To determine the mechanism of MAO inhibition by the antidepressants and mood stabilizers, the effects of various concentrations of these drugs on MAO kinetic were studied. Enzyme kinetic equations for competitive, non-competitive, uncompetitive and mixed inhibition type were tested and the best model was chosen.

When the kinetics of the interactions of drugs with the enzyme were assessed, SigmaPlot calculation of MAO-A kinetics confirmed that the interaction was competitive for moclobemide and olanzapine only (i.e. apparent increases in K_m values with no changes in V_{\max} values in the presence of the inhibitor), non-competitive for fluoxetine, citalopram, venlafaxine, reboxetine and mirtazapine (i.e. apparent decreases in

V_{\max} values with no changes in K_m values in the presence of the inhibitor), mixed for desipramine, imipramine and amitriptyline (i.e. apparent decreases in V_{\max} values with the change in K_m values in the presence of the inhibitor), and uncompetitive for tianeptine (i.e. apparent decreases in V_{\max} values with decrease in K_m values in the presence of the inhibitor) (Table 1).

The effects of the same drugs also were evaluated on kinetic parameters of pig brain mitochondrial MAO-B activity. SigmaPlot calculation of MAO-B kinetics confirmed that the interaction was non-competitive for olanzapine and mirtazapine, mixed for fluoxetine, amitriptyline, desipramine, imipramine and reboxetine, and uncompetitive for venlafaxine, citalopram and tianeptine (Table 2).

Both lithium and valproate are very weak MAOIs; mechanism of MAO-A or MAO-B inhibition at very high drug concentrations appears to be non-competitive. Cocaine is weak MAOs inhibitor with mixed type of inhibition.

Competitive inhibition of MAOs by drugs means that their inhibition constants K_i must be calculated using Cheng-Prusoff equation; however, K_i is equal to IC_{50} (see the Tables 1 and 2) under conditions of either non-competitive or uncompetitive kinetics.

Table 2 Inhibition of MAO-B activity in brain crude mitochondrial fraction

	IC_{50} μ mol/l	Hill slope	Mechanism of inhibition	N
Fluoxetine	35.7 \pm 3.1	1.23 \pm 0.12	Mixed	4
Venlafaxine	48.0 \pm 4.8	1.26 \pm 0.13	Uncompetitive	10
Amitriptyline	72.1 \pm 8.0	1.41 \pm 0.19	Mixed	8
Olanzapine	79 \pm 44	0.66 \pm 0.21	Non-competitive	9
Citalopram	110 \pm 11	0.975 \pm 0.089	Uncompetitive	5
Desipramine	140 \pm 18	0.859 \pm 0.085	Mixed	7
Reboxetine	364 \pm 81	0.93 \pm 0.14	Mixed	7
Imipramine	452 \pm 51	1.006 \pm 0.098	Mixed	5
Tianeptine	526 \pm 34	1.65 \pm 0.14	Uncompetitive	7
Mirtazapine	546 \pm 108	0.83 \pm 0.12	Non-competitive	15
Moclobemide	>10000			7
Lithium	>10000			6
Valproate	>10000			6
Pargyline	0.00820 \pm 0.00033	1.311 \pm 0.057	Irreversible	11
Clorgyline	0.346 \pm 0.019	1.118 \pm 0.065	Irreversible	6
Iproniazid	1.210 \pm 0.067	1.156 \pm 0.064	Irreversible	10
Cocaine	543 \pm 231	0.77 \pm 0.20	Mixed	6

The samples were incubated with drugs at 37 °C for 60 minutes and the reaction was started by addition of 10 μ mol/l [14 C] phenylethylamine; following incubation at 37 °C for 1 minute, the reaction was stopped by addition of hydrochloric acid. The samples were measured in doublets and blank values were deducted. Values are means \pm standard deviation. IC_{50} = drug concentration that is required for 50% inhibition of enzyme activity; Hill slope = describes the cooperativity of the drug binding; N = number of measurements.

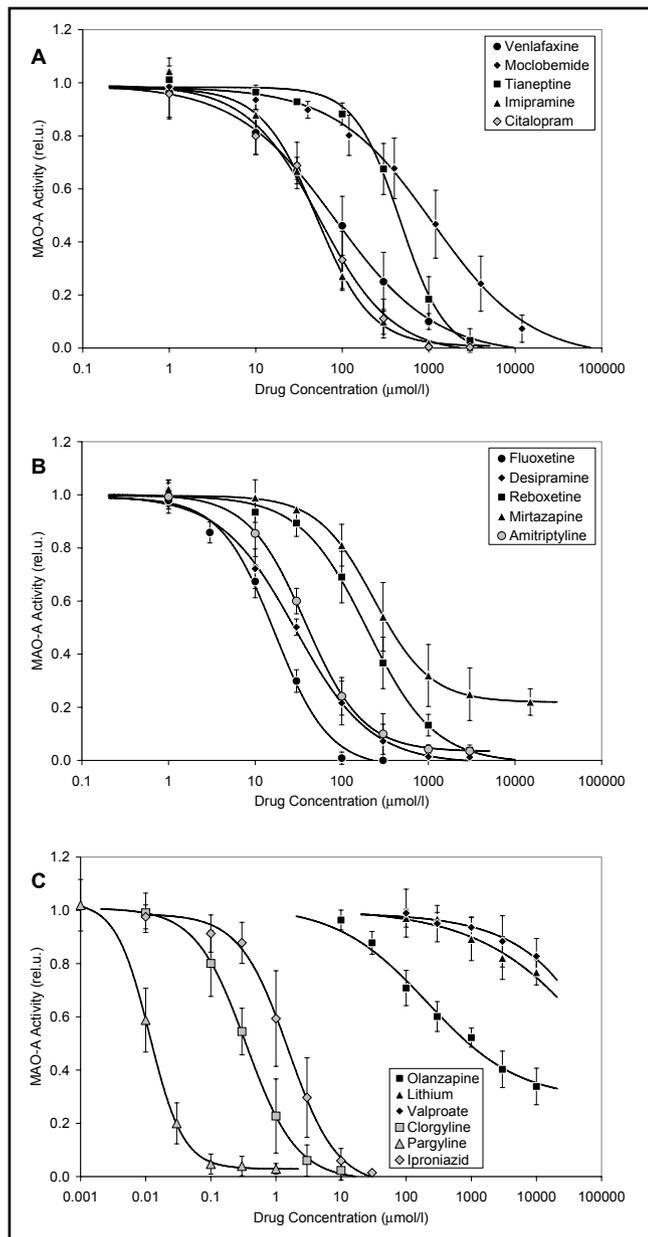


Fig. 1. Inhibition of basal MAO-A activity by antidepressants in crude mitochondrial fraction isolated from cortex of pig brain. Dose-response curves are displayed as plots of the initial activity of MAO-A against the antidepressant concentration. The samples were incubated with drugs at 37 °C for 60 minutes and the reaction was started by the addition of 3.2 μmol/l [¹⁴C]serotonin. Following incubation at 37 °C for 30 minutes, the reaction was stopped by the addition of hydrochloric acid. The samples were measured in doublets and blank values were deducted. Median effective concentrations (IC_{50}) were calculated using nonlinear regression analysis software (Table 1). Values are means ± standard deviation. Lines represent the best fitted curves using the four-parameter logistic function.

DISCUSSION

The current study demonstrates that pharmacologically different antidepressants can inhibit MAO activity in mitochondrial preparations from brain tissue, modulating the monoaminergic systems in this way.

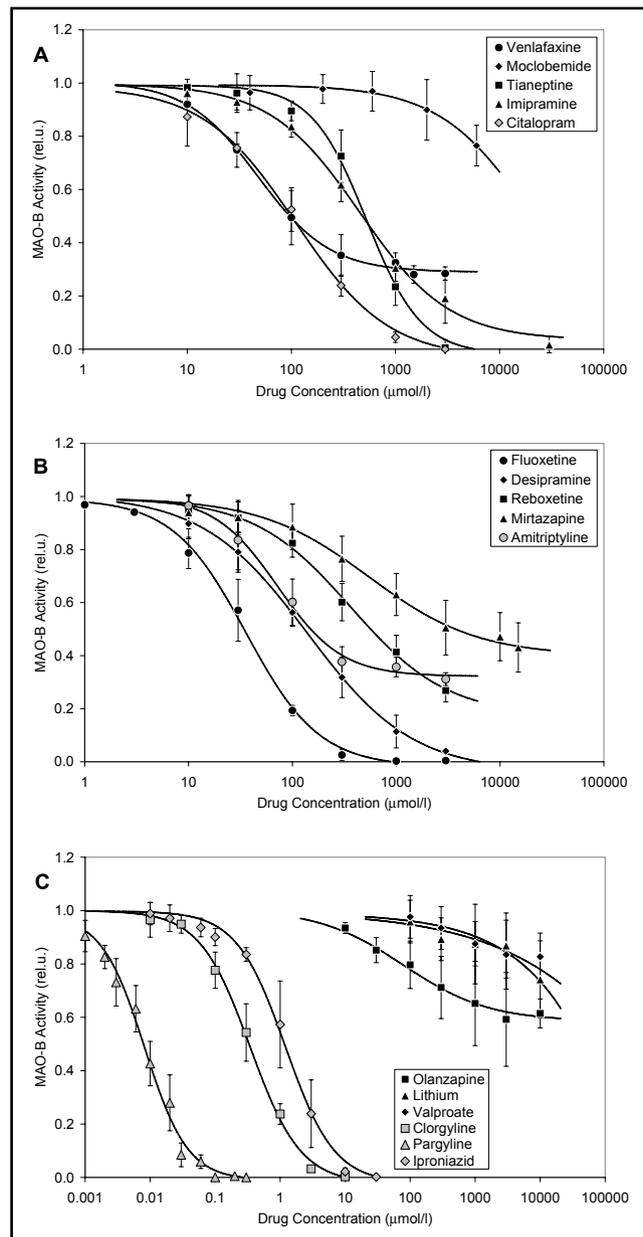


Fig. 2. Inhibition of basal MAO-B activity by antidepressants in crude mitochondrial fraction isolated from cortex of pig brain. Dose-response curves are displayed as plots of the initial activity of MAO-B against the antidepressant concentration. The samples were incubated with drugs at 37 °C for 60 minutes and the reaction was started by the addition of 10 μmol/l [¹⁴C]phenylethylamine. Following incubation at 37 °C for 1 minute, the reaction was stopped by the addition of hydrochloric acid. The samples were measured in doublets and blank values were deducted. Median effective concentrations (IC_{50}) were calculated using nonlinear regression analysis software (Table 2). Values are means ± standard deviation. Lines represent the best fitted curves using the four-parameter logistic function.

Experimental conditions of the present study, use of the selective substrates 5-HT for MAO-A and PEA for MAO-B, allowed the inhibitory effects and mechanism of the interactions of antidepressants on the two MAO isoforms to be evaluated separately. The potency, isoenzymes selectivity and mechanism of inhibition of repre-

sentative antidepressants and mood stabilizers towards pig brain MAO were assessed.

Enzyme inhibition represents one of the most common strategies in the development of therapeutic drug candidates. Estimation of an enzyme inhibitor binding affinity is an important step in predicting *in vivo* potency, selectivity, and potential for metabolic interactions. The reliability of K_i as an indicator of *in vivo* potency and selectivity is obviously dependent on the accuracy and precision of its *in vitro* estimation. Hypothesis was tested that the pharmacologically different antidepressants or mood stabilizers could act, at least in part, by inhibition of MAO.

Our study confirmed and extended previous results (Edwards & Burns 1974; Green & McGachy 1987; Reid *et al.* 1988; Egashira *et al.* 1996, 1999; Gnerre *et al.* 2001) that antidepressant drugs of various pharmacological classes (TCA, SSRI, NRI, SNRI, NaSSA, NDRI, SSRE) show inhibitory effects on MAOs. All tested antidepressants and mood stabilizers, except for venlafaxine and olanzapine, were more potent inhibitors of MAO-A than MAO-B (ratio of IC_{50} for MAO-A to IC_{50} for MAO-B was lower than 0.1 for moclobemide, and varied from 0.11 for imipramine to 0.89 for tianeptine). Different IC_{50} or K_i values and mechanisms of inhibition by specific antidepressant were described in various studies; it could be explained both by different experimental conditions and by diverse data evaluation techniques. In accordance with previous studies (Mukherjee & Yang 1997, 1999; Gnerre *et al.* 2001) we found lower affinity of citalopram for MAOs compared to fluoxetine. We observed that olanzapine (antipsychotic and mood stabilizing drug) exhibit similar inhibitory effect on MAOs as antidepressants. Direct *in vitro* inhibitory effect of lithium and valproate was confirmed to be insignificant (Nag 2004).

We established that moclobemide is a relatively weak MAO-A inhibitor *in vitro*; the dissociation constant of the enzyme-inhibitor complex was found in the range of hundreds of $\mu\text{mol/l}$ (Kettler *et al.* 1990; Cesura *et al.* 1992). In spite of its weak MAO-A inhibition *in vitro*, moclobemide is a potent inhibitor of MAO-A *in vivo* (Burkard *et al.* 1989; Da Prada *et al.* 1990); plasma concentrations associated with 50% of maximum enzyme activity were found about of one $\mu\text{mol/l}$ (Holford *et al.* 1994; Guentert *et al.* 1995). This discrepancy suggested that the compound might be converted *in vivo* to an active form (Kettler *et al.* 1990). Reversibility of MAO-A inhibition was demonstrated *in vitro* as well as *in vivo* (Haefely *et al.* 1993). *In vitro* investigations of kinetics of inhibition of MAO-A by moclobemide have shown that inhibition is time-dependent. Initially, the inhibition is competitive, changing gradually to become non-competitive (Cesura *et al.* 1992; Nair *et al.* 1993). We observed competitive inhibition of MAO-A by moclobemide in our experimental conditions.

In the present study all drugs tested inhibited MAOs at higher concentrations. These concentrations were

much higher than therapeutically active plasma concentrations of antidepressants *in vivo*. However, there is accumulation of psychotropic drugs in the brain and the brain levels of antidepressants are a reflection not only of their plasma concentrations, but also of their distribution between plasma and red blood cells (Fišar *et al.* 1996; Fišar *et al.* 2006). E.g. the apparent concentration of fluoxetine in brain relative to plasma was found 20:1. The brain concentrations of fluoxetine/norfluoxetine were found to reach for up to 35 $\mu\text{mol/l}$ (Karson *et al.* 1993). It has also been shown that fluoxetine is present in subcellular components of the rodent brain, including mitochondria and synaptosomes (Caccia *et al.* 1990). Thus, with high subcellular concentrations and micromolar affinities for MAO, fluoxetine has the potential to exert a significant inhibitory effect on the enzyme. Consequently, the possibility cannot be excluded that MAO activity is affected due to long lasting action of amphiphilic antidepressants and their accumulation in mitochondrial membrane during chronic treatment.

Since MAO is embedded in the outer mitochondrial membrane, lipid-protein interactions play a role in the functional properties of MAOs (Huang & Faulkner 1981; Fowler *et al.* 2007), and antidepressants are capable of changing membrane fluidity, it can be speculated about a role of accumulation of antidepressants in the lipid bilayer related to their effect on MAO activity. However, some data indicate that fluidity modulation is not the cause of MAO inhibition (Muriel & Pérez-Rojas 2003).

The analysis showed that mechanism of inhibition of MAOs by antidepressants may differ with respect both to different drug molecules and to MAO substrates. Competitive inhibition was found for moclobemide and olanzapine only when 5-HT was used as substrate. Competitive inhibition would be consistent with drug binding to the active sites of the MAO-A. Desipramine, amitriptyline, imipramine, mirtazapine and tianeptine retain the same mechanism of inhibition for both MAO-A and MAO-B.

Inhibitory and kinetic parameters calculated in this paper result from total concentrations of drugs added to crude brain mitochondrial fraction. Situation in various tissues *in vivo* may be different from our experimental conditions. This was reason why the effects of commonly used antidepressants and mood stabilizers on MAOs activity were compared with effects of well-known MAO inhibitors such as iproniazid, pargyline and clorgyline. The results shown that all tested antidepressants are much weaker MAOs inhibitors compared to those irreversible MAOIs. Contrary, cocaine, which significantly raises the amount of extraneuronal dopamine and serotonin in the brain, although is not antidepressant, is very weak inhibitor of MAOs.

It was suggested that inhibition of brain MAO could contribute to slowing, halting and maybe reversing of neurodegeneration (e.g. in dopaminergic neurons),

which was initiated by oxidative stress (Youdim *et al.* 2006). Neuroprotection has been uncovered as common effect of long-term treatment of mood disorders and Parkinson's disease with different antidepressants, mood stabilizers and antipsychotics. The molecular mechanism of neuroprotection is still unclear; however, it is obvious that reduction of local oxidative stress is a major component, and MAO inhibition participates in this reduction.

An unanswered question in the field pharmacotherapy of mental disorders remains why some individuals are more vulnerable than others to substance use disorders or to therapeutic effects of psychotropic drugs. Differences both in drug metabolism and in activity of neurotransmitter systems are included in interindividual differences in response to drug treatment. Antidepressants and other widely used drugs are also unlabeled MAO inhibitors. Exact physiological role of MAO inhibition by tested antidepressants (except for moclobemide) is unknown; however, the effects of drugs on MAO activity may contribute to their modulation of monoamine neurotransmission in the brain. It can be speculated that inhibition of MAO by pharmacologically different antidepressants could contribute to the regulation of mood and emotions or to the interindividual differences in the drug response.

Generally, pharmacologically different antidepressants were found to be rather weak MAO inhibitors. There is evident discrimination both among several drugs and the two MAO isoforms. Because *in vitro* inhibition of MAOs by antidepressants was demonstrated and there is drug accumulation in the brain tissue, hypothesis was supported that *in vivo* inhibition of MAO by pharmacologically different antidepressants would amplify their effects on serotonergic, noradrenergic and dopaminergic neurotransmission. Although it is always difficult to extrapolate from *in vitro* studies to the clinical reality, the present results suggest that inhibitory effect of antidepressants on MAO activity can not be neglected.

ACKNOWLEDGEMENTS

This research was supported by grant No MSM0021620849 given by Ministry of Education, Youth and Sports of the Czech Republic and by pharmaceutical company Zentiva Group, a.s. Praha. The author would like to thank Zdeněk Hanuš for his technical assistance.

REFERENCES

- Akaike H (1974). A new look at the statistical model identification. *IEEE Transactions on Automatic Control*. **19**: 716–723.
- Bach AWJ, Lan NC, Johnson DL, Abell CW, Bembenek ME, Kwan S-W, et al (1988). cDNA cloning of human liver monoamine oxidase A and B: molecular basis of differences in enzymatic properties. *Proc Natl Acad Sci USA*. **85**: 4934–4938.
- Baker GB, Urchuk LJ, McKenna KF, Kennedy SH (1999). Metabolism of monoamine oxidase inhibitors. *Cell Mol Neurobiol*. **19**: 411–426.
- Bonnet U (2003). Moclobemide: therapeutic use and clinical studies. *CNS Drug Rev*. **9**: 97–140.
- Burkard WP, Da Prada M, Keller HH, Kettler R, Haefely W (1989). Pre-clinical pharmacology of moclobemide. A review of published studies. *Br J Psychiatry*. **154**(Suppl 6): 84–88.
- Caccia S, Cappi M, Fracasso C, Garattini S (1990). Influence of dose and route of administration on the kinetics of fluoxetine and its metabolite norfluoxetine in the rat. *Psychopharmacology (Berl)*. **100**: 509–514.
- Cesura AM, Kettler R, Imhof R, Da Prada M (1992). Mode of action and characteristics of monoamine oxidase-A inhibition by moclobemide. *Psychopharmacology (Berl)*. **106**(Suppl 1): S15–S16.
- Chen ML, Chen CH (2007). Chronic antipsychotics treatment regulates MAOA, MAOB and COMT gene expression in rat frontal cortex. *J Psychiatr Res*. **41**: 57–62.
- Chen K, Wu HF, Shih JC (1993). The deduced amino acid sequences of human platelet and frontal cortex monoamine oxidase B are identical. *J Neurochem*. **61**: 187–190.
- Cheng Y-C, Prusoff WH (1973). Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. *Biochem Pharmacol*. **22**: 3099–3108.
- Da Prada M, Kettler R, Keller HH, Burkard WP, Muggli-Maniglio D, Haefely WE (1989). Neurochemical profile of moclobemide, a short-acting and reversible inhibitor of monoamine oxidase type A. *J Pharmacol Exp Ther*. **248**: 400–414.
- Da Prada M, Kettler R, Keller HH, Cesura AM, Richards JG, Saura Marti J, et al (1990). From moclobemide to Ro 19-6327 and Ro 41-1049: the development of a new class of reversible, selective MAO-A and MAO-B inhibitors. *J Neural Transm Suppl*. **29**: 279–292.
- Davidson J, Mcleod MN, Linnoila M, Kurland AA, White HL (1978). Platelet MAO inhibition following tricyclic antidepressant therapy. *Am J Psychiatry*. **135**: 603–605.
- Edmondson DE, Binda C, Wang J, Upadhyay AK, Mattevi A (2009). Molecular and mechanistic properties of the membrane-bound mitochondrial monoamine oxidases. *Biochemistry*. **48**: 4220–4230.
- Edwards DJ, Burns MO (1974). Effects of tricyclic antidepressants upon human platelet monoamine oxidase. *Life Sci*. **15**: 2045–2058.
- Egashira T, Kuroiwa Y, Kamijo K (1978). Multiple catalytic sites of rat brain mitochondrial monoamine oxidase. *Arch Biochem Biophys*. **191**: 714–718.
- Egashira T, Takayama F, Yamanaka Y (1996). Effects of long-term treatment with dicyclic, tricyclic, tetracyclic, and noncyclic antidepressant drugs on monoamine oxidase activity in mouse brain. *Gen Pharmacol*. **27**: 773–778.
- Egashira T, Takayama F, Yamanaka Y (1999). The inhibition of monoamine oxidase activity by various antidepressants: differences found in various mammalian species. *Jpn J Pharmacol*. **81**: 115–121.
- Ekstedt B (1976). Substrate specificity of the different forms of monoamine oxidase in rat liver mitochondria. *Biochem Pharmacol*. **25**: 1133–1138.
- Fagervall I, Ross SB (1986). Inhibition of monoamine oxidase in monoaminergic neurones in the rat brain by irreversible inhibitors. *Biochem Pharmacol*. **35**: 1381–1387.
- Fišar Z, Krulík R, Fuksová K, Sikora J (1996). Imipramine distribution among red blood cells, plasma and brain tissue. *Gen Physiol Biophys*. **15**: 51–64.
- Fišar Z, Fuksová K, Sikora J, Kališová L, Velenovská M, Novotná M (2006). Distribution of antidepressants between plasma and red blood cells. *Neuroendocrinol Lett*. **27**: 307–313.
- Fišar Z, Raboch J (2008). Depression, antidepressants, and peripheral blood components. *Neuroendocrinol Lett*. **29**: 17–28.
- Fišar Z, Hroudová J (2010). Intracellular signalling pathways and mood disorders. *Folia Biol*. **56**: 135–148.

- 25 Fowler CJ, Tipton KF (1981). Concentration dependence of the oxidation of tyramine by the two forms of rat liver mitochondrial monoamine oxidase. *Biochem Pharmacol.* **30**: 3329–3332.
- 26 Fowler PW, Balali-Mood K, Deol S, Coveney PV, Sansom MS (2007). Monotopic enzymes and lipid bilayers: a comparative study. *Biochemistry.* **46**: 3108–3115.
- 27 Gnerre C, Kosel M, Baumann P, Carrupt P-A, Testa B (2001). Interaction of psychotropic drugs with monoamine oxidase in rat brain. *J Pharm Pharmacol.* **53**: 1125–1130.
- 28 Gómez N, Balsa D, Unzeta M (1988). A comparative study of some kinetic and molecular properties of microsomal and mitochondrial monoamine oxidase. *Biochem Pharmacol.* **37**: 3407–3413.
- 29 Green AL, McGachy HA (1987). The inhibition of monoamine oxidase by tricyclic antidepressants: the influence of the nature of the substrate and the source of the enzyme. *J Pharm Pharmacol.* **39**: 392–394.
- 30 Green AL, O'Grady JE, Vass M (1989). The effect of some tricyclic antidepressants on the inhibition of mouse brain monoamine oxidase in-vivo by phenelzine. *J Pharm Pharmacol.* **41**: 50–51.
- 31 Guentert TW, Banken L, Hilton S, Holford NH (1995). Moclobemide: relationships between dose, drug concentration in plasma, and occurrence of adverse events. *J Clin Psychopharmacol.* **15**(Suppl 2): 845–945.
- 32 Haddjeri N, Szabo ST, de Montigny C, Blier P (2000). Increased tonic activation of rat forebrain 5-HT_{1A} receptors by lithium addition to antidepressant treatments. *Neuropsychopharmacology.* **22**: 346–356.
- 33 Haefely W, Burkard WP, Cesura AM, Kettler R, Lorez HP, Martin JR, et al (1992). Biochemistry and pharmacology of moclobemide, a prototype RIMA. *Psychopharmacology (Berl).* **106**(Suppl 1): S6–S14.
- 34 Haefely W, Burkard WP, Cesura A, Colzi A, Kettler R, Lorez HP, et al (1993). Pharmacology of moclobemide. *Clin Neuropharmacol.* **16**(Suppl 2): S8–S18.
- 35 Hindmarch I (2002). Beyond the monoamine hypothesis: mechanisms, molecules and methods. *Eur Psychiatry.* **17**(Suppl 3): 294–299.
- 36 Holford NH, Guentert TW, Dingemans J, Banken L (1994). Monoamine oxidase-A: pharmacodynamics in humans of moclobemide, a reversible and selective inhibitor. *Br J Clin Pharmacol.* **37**: 433–439.
- 37 Honecker H, Christ W, Müller-Oerlinghausen B, Coper H (1976). Critical evaluation of measurement of platelet monoamine oxidase in man. *J Clin Chem Clin Biochem.* **14**: 453–458.
- 38 Horstink M, Tolosa E, Bonuccelli U, Deuschl G, Friedman A, Kanovsky P, et al; European Federation of Neurological Societies; Movement Disorder Society-European Section (2006). Review of the therapeutic management of Parkinson's disease. Report of a joint task force of the European Federation of Neurological Societies and the Movement Disorder Society-European Section. Part I: early (uncomplicated) Parkinson's disease. *Eur J Neurol.* **13**: 1170–1185.
- 39 Huang RH, Faulkner R (1981). The role of phospholipid in the multiple functional forms of brain monoamine oxidase. *J Biol Chem.* **256**: 9211–9215.
- 40 Iwersen S, Schmoldt A (1996). Three suicide attempts with moclobemide. *J Toxicol Clin Toxicol.* **34**: 223–225.
- 41 Jauch R, Griesser E, Oesterhelt G, Arnold W, Meister W, Ziegler WH, et al (1990). Biotransformation of moclobemide in humans. *Acta Psychiatr Scand.* **82**(Suppl 360): 87–90.
- 42 Karson CN, Newton JE, Livingston R, Jolly JB, Cooper TB, Sprigg J, et al (1993). Human brain fluoxetine concentrations. *J Neuropsychiatry Clin Neurosci.* **5**: 322–329.
- 43 Kessler RC, Angermeyer M, Anthony JC, DE Graaf R, Demyttenaere K, Gasquet I, et al (2007). Lifetime prevalence and age-of-onset distributions of mental disorders in the World Health Organization's World Mental Health Survey Initiative. *World Psychiatry.* **6**: 168–176.
- 44 Kettler R, Da Prada M, Burkard WP (1990). Comparison of monoamine oxidase-A inhibition by moclobemide *in vitro* and *ex vivo* in rats. *Acta Psychiatr Scand.* **82**(Suppl 360): 101–102.
- 45 Kitaichi Y, Inoue T, Nakagawa S, Izumi T, Koyama T (2006). Effect of co-administration of subchronic lithium pretreatment and acute MAO inhibitors on extracellular monoamine levels and the expression of contextual conditioned fear in rats. *Eur J Pharmacol.* **532**: 236–245.
- 46 Lecrubier Y, Gueffi JD (1990). Efficacy of reversible inhibitors of monoamine oxidase-A in various forms of depression. *Acta Psychiatr Scand.* **82**(Suppl 360): 18–23.
- 47 Lecrubier Y (1994). Risk-benefit assessment of newer versus older monoamine oxidase (MAO) inhibitors. *Drug Saf.* **10**: 292–300.
- 48 Lotufo-Neto F, Trivedi M, Thase ME (1999). Meta-analysis of the reversible inhibitors of monoamine oxidase type A moclobemide and brofaromine for the treatment of depression. *Neuropsychopharmacology.* **20**: 226–247.
- 49 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the Folin phenol reagent. *J Biol Chem.* **193**: 265–275.
- 50 Magder DM, Aleksic I, Kennedy SH (2000). Tolerability and efficacy of high-dose moclobemide alone and in combination with lithium and trazodone. *J Clin Psychopharmacol.* **20**: 394–395.
- 51 Meyer JH, Ginovart N, Boovariwala A, Sagrati S, Hussey D, Garcia A et al (2006). Elevated monoamine oxidase levels in the brain: an explanation for the monoamine imbalance of major depression. *Arch Gen Psychiatry.* **63**: 1209–1216.
- 52 Mukherjee J, Yang Z-Y (1997). Evaluation of monoamine oxidase B inhibition by fluoxetine (Prozac): an *in vitro* and *in vivo* study. *Eur J Pharmacol.* **337**: 111–114.
- 53 Mukherjee J, Yang Z-Y (1999). Monoamine oxidase A inhibition by fluoxetine: an *in vitro* and *in vivo* study. *Synapse.* **31**: 285–289.
- 54 Muraki I, Inoue T, Hashimoto S, Izumi T, Ito K, Koyama T (2001). Effect of subchronic lithium treatment on citalopram-induced increases in extracellular concentrations of serotonin in the medial prefrontal cortex. *J Neurochem.* **76**: 490–497.
- 55 Muriel P, Pérez-Rojas JM (2003). Nitric oxide inhibits mitochondrial monoamine oxidase activity and decreases outer mitochondrial membrane fluidity. *Comp Biochem Physiol C Toxicol Pharmacol.* **136**: 191–197.
- 56 Nag M (2004). Effect of chlorpromazine, imipramine and lithium on MAO-A and MAO-B activity in rat brain mitochondria. *Indian J Exp Biol.* **42**: 941–944.
- 57 Nair NPV, Ahmed SK, Kin NMK (1993). Biochemistry and pharmacology of reversible inhibitors of MAO-A agents: focus on moclobemide. *J Psychiatr Neurosci.* **18**: 214–225.
- 58 Nelson JC (1999). A review of the efficacy of serotonergic and noradrenergic reuptake inhibitors for treatment of major depression. *Biol Psychiatry.* **46**: 1301–1308.
- 59 Nierenberg AA, Fava M, Trivedi MH, Wisniewski SR, Thase ME, McGrath PJ, et al (2006). A comparison of lithium and T₃ augmentation following two failed medication treatments for depression: a STAR*D report. *Am J Psychiatry.* **163**: 1519–1530.
- 60 Ozaita A, Olmos G, Boronat MA, Lizcano JM, Unzeta M, García-Sevilla JA (1997). Inhibition of monoamine oxidase A and B activities by imidazol(ine)/guanidine drugs, nature of the interaction and distinction from I₂-imidazoline receptors in rat liver. *Br J Pharmacol.* **121**: 901–912.
- 61 Papakostas GI, Thase ME, Fava M, Nelson JC, Shelton RC (2007). Are antidepressant drugs that combine serotonergic and noradrenergic mechanisms of action more effective than the selective serotonin reuptake inhibitors in treating major depressive disorder? A meta-analysis of studies of newer agents. *Biol Psychiatry.* **62**: 1217–1227.
- 62 Praško J, Dockery C, Horáček J, Houbová P, Kosová J, Klaschka J, et al (2006). Moclobemide and cognitive behavioral therapy in the treatment of social phobia. A six-month controlled study and 24 months follow up. *Neuroendocrinol Lett.* **27**: 473–481.
- 63 Ramsay RR, Gravestock MB (2003). Monoamine oxidases: to inhibit or not to inhibit. *Mini Rev Med Chem.* **3**: 129–136.
- 64 Redrobe JP, Bourin M (1999). Evidence of the activity of lithium on 5-HT_{1B} receptors in the mouse forced swimming test: comparison with carbamazepine and sodium valproate. *Psychopharmacology (Berl).* **141**: 370–377.

- 65 Reid AA, Hill JL, Murphy DL (1988). Interactions of tricyclic antidepressants with human and rat monoamine oxidase type B. *Naunyn Schmiedeberg Arch Pharmacol.* **338**: 678–83.
- 66 Reveley MA, Glover V, Sandler M, Coppen A (1979). Absence of platelet monoamine oxidase inhibition during amitriptyline or zimelidine treatment. *Br J Clin Pharmacol.* **8**: 375–378.
- 67 Reveley MA, Glover V, Sandler M, Coppen A (1981). Increased platelet monoamine oxidase activity in affective disorders. *Psychopharmacology (Berl).* **73**: 257–260.
- 68 Reyes E, Lisansky J (1984). Effects of tricyclic antidepressants on platelet monoamine oxidase activity. *Clin Pharmacol Ther.* **35**: 531–534.
- 69 Riederer P, Danielczyk W, Grünblatt E (2004). Monoamine oxidase-B inhibition in Alzheimer's disease. *NeuroToxicology.* **25**: 271–277.
- 70 Roth JA, Gillis CN (1974). Inhibition of lung, liver and brain monoamine oxidase by imipramine and desipramine. *Biochem Pharmacol.* **23**: 1138–1140.
- 71 Smith TE, Weissbach H, Udenfriend S (1963). Studies on monoamine oxidase: the mechanism of inhibition of monoamine oxidase by iproniazid. *Biochemistry.* **2**: 746–751.
- 72 Sullivan JL, Dackis C, Stanfield C (1977). *In vivo* inhibition of platelet MAO activity by tricyclic antidepressants. *Am J Psychiatry.* **134**: 188–190.
- 73 Sullivan JL, Zung WW, Stanfield CN, Cavenar JO Jr (1978). Clinical correlates of tricyclic antidepressant-mediated inhibition of platelet monoamine oxidase. *Biol Psychiatry.* **13**: 399–407.
- 74 Stahl SM, Felker A (2008). Monoamine oxidase inhibitors: a modern guide to an unrequited class of antidepressants. *CNS Spectr.* **13**: 855–870.
- 75 Ulus IH, Maher TJ, Wurtman RJ (2000). Characterization of phen-termine and related compounds as monoamine oxidase (MAO) inhibitors. *Biochem Pharmacol.* **59**: 1611–1621.
- 76 Whittaker VP (1969). The synaptosome. In: Lajtha A, editor. *Handbook of Neurochemistry. Vol. II Structural Neurochemistry.* New York, London: Plenum Press. p. 327–364.
- 77 Youdim MB, Bakhle YS (2006). Monoamine oxidase: isoforms and inhibitors in Parkinson's disease and depressive illness. *Br J Pharmacol.* **147**(Suppl 1): S287–S296.
- 78 Youdim MB, Edmondson D, Tipton KF (2006). The therapeutic potential of monoamine oxidase inhibitors. *Nat Rev Neurosci.* **7**: 295–309.