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Interaction of psychotropic drugs with monoamine oxidase in rat brain

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Abstract

Literature observations indicate that some psychotropic drugs may have inhibitory activity towards monoamine oxidase (MAO). This study was undertaken to assess the potency, isozyme selectivity and mechanism of inhibition of representative first- and second-generation antidepressant drugs towards rat brain MAO-A and MAO-B. Five tricyclic antidepressants (imipramine, trimipramine, clomipramine, amitriptyline and doxepine) and three selective serotonin reuptake inhibitors (fluoxetine, fluvoxamine and citalopram) were examined. They showed inhibitory activity towards MAO-A and MAO-B, with clear selectivity for MAO-B (K_i in the micromolar range). Their mechanism of inhibition was competitive towards MAO-B and of a mixed competitive type towards MAO-A. The results suggest that some of the drugs examined might also contribute an MAO inhibitory effect in chronically treated patients.

Introduction

Impaired monoamine pathways, particularly of noradrenaline and serotonin (5-hydroxytryptamine) is one of the current hypotheses accounting, in part, for depressive illness (Mukherjee & Yang 1999). Effective drug treatments for depression have been available for more than three decades. First-generation tricyclic antidepressants, even when eliciting anticholinergic and cardiovascular side-effects, are still in use, probably due to lower costs. Second-generation antidepressants tend to be more selective in their activity and atypical in structure, and generally lack the characteristic side-effects of first-generation drugs (Broekkamp et al 1995).

A substantial minority of patients fail to respond adequately to antidepressant treatment (Nierenberg & Amsterdam 1990; Sokolov & Russell 1995; Souery et al 1999). One strategy to deal with refractory or resistant depression includes substitution with an alternative antidepressant or a combination of drugs, for example, tricyclic antidepressants with monoamine oxidase (MAO) inhibitors. However, the combination of MAO inhibitors with selective serotonin reuptake inhibitors (SSRIs) or with the tricyclic antidepressant clomipramine, selective towards blockade of serotonin reuptake, must be avoided due to the high incidence of a serotonin syndrome (Peyman & Beckhaus 1992; Sokolov & Russell 1995).

It has been demonstrated that tricyclic antidepressants, such as imipramine, have an inhibitory activity towards MAO and particularly MAO-B (Green & McGachy 1987). However, systematic studies are lacking. The aim of this work was, therefore,

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to determine the in-vitro inhibitory activity of a series of first- and second-generation antidepressants towards MAO-A and MAO-B. The mechanism by which these compounds inhibited the oxidative metabolism of kynuramine, a non-selective MAO-A and MAO-B substrate, was assessed by complete enzyme kinetics. The results are discussed in relation to possible clinical implications.

Materials and Methods

Chemicals

The following products were obtained from commercial sources and used without further purification: Na_2HPO_4, KCl, KH_2PO_4, dimethylsulfoxide (DMSO) and sucrose (Fluka Chemie AG, Buchs, CH); kynuramine, clorgyline, imipramine, clomipramine, trimipramine (Sigma Chemical Co., St Louis, MO); amitriptyline (Aldrich Chemical Company, Milwaukee, WI); doxepine, fluvoxamine, fluoxetine (Tocris, Bristol, UK); (−)-deprenyl (RBI, Natick, MA). The following drugs were gifts: the enantiomers of citalopram (R-CIT and S-CIT), the racemates of CIT, desmethylcitalopram (desmethyl-CIT) and didesmethylcitalopram (didesmethyl-CIT) (Lundbeck AS, Copenhagen, Denmark).

Rat brain mitochondria isolation

The method used for rat brain mitochondria isolation was in strict accordance with the Swiss legislation and the "Ethical principles and guidelines for experiments on animals" published by the Swiss Academy of Medical Sciences and the Swiss Academy of Sciences (1993). Sprague-Dawley rats, 200–250 g, (RCC, Füllinsdorf, CH) were anaesthetized in a mixture of CO_2 and air for about 40 s and then decapitated. Rat brain mitochondria were isolated according to the method of Clark & Nicklas (1970) modified by Walther et al (1987). The blood vessels and pial membranes were carefully removed, and the brains homogenized in buffer (Na_2HPO_4/0.15 M KH_2PO_4 buffer, pH 7.4, isotonized with 0.13 M sucrose) with a Dounce tissue grinder (Wheaton, Millville, NJ). Homogenates were first centrifuged (Centrikon T-2070, rotor type TFT6538; Kontron AG, CH) at 1900 *g* to remove nuclear pellets. Nuclear pellets were washed once, and the supernatants were then mixed and centrifuged at 11 800 *g*. The crude mitochondrial pellet obtained was washed once again with buffer and then resuspended in buffer and aliquoted in 1-mL portions to be stored at −80°C. Protein concentration was determined according to Lowry et al (1951) and was set to a final value of 1.0 mg mL^{-1} in the assay mixture.

Measurement of MAO inhibitory activity

Incubations were carried out in a pH 7.4 buffer (Na_2HPO_4/KH_2PO_4) made isotonic with KCl, in the presence of rat brain mitochondria. The protein suspension was pre-incubated at 37°C for 5 min with either clorgyline (250 nM) or (−)-deprenyl (250 nM) to test for MAO-B or MAO-A activity, respectively (Thull et al 1993). The inhibitors under study (Figure 1) were solubilized in DMSO and added to the suspension to give a final concentration of cosolvent of 5% (v/v), a concentration that did not affect MAO activity. The non-selective substrate, kynuramine, was then added. Kynuramine is deaminated by MAO to an aldehyde that spontaneously cyclizes to 4-hydroxyquinoline, a strongly UV-active product the formation of which was monitored at 314 nm using a Kontron UVIKON 941 spectrophotometer.

Determination of constants of inhibition

IC50 values were determined according to a general hyperbolic model as already described (Thull et al 1993) using kynuramine at a concentration corresponding to its K_m (Michaelis constant) value for MAO-A (90 μM) and MAO-B (60 μM). Incubations were carried out in duplicate at each inhibitor concentration; the degrees of inhibition, so obtained, were fed into the software GraphPad Prism ver. 3.02, which used the hyperbolic model to calculate the IC50 values and the standard error. When solubility was not high enough to reach the IC50, the percentage inhibition at the highest concentration tested was reported instead. The effect of pre-incubation time was evaluated by pre-incubating the compounds under study for 15 min instead of 5 min, and comparing the degrees of inhibition.

To determine the mechanism of inhibition, the assays were carried out at different kynuramine concentrations and without or with two different concentrations of drug. Kynuramine concentrations ranged from 60 to 270 μM for MAO-A and from 30 to 180 μM for MAO-B. Incubations were run in duplicate and Lineweaver-Burk double reciprocal plots were calculated by linear regression using the GraphPad Prism software. The kinetic constants of competitive inhibition (K_i) were determined from IC50 values using the Cheng-Prusoff equation,

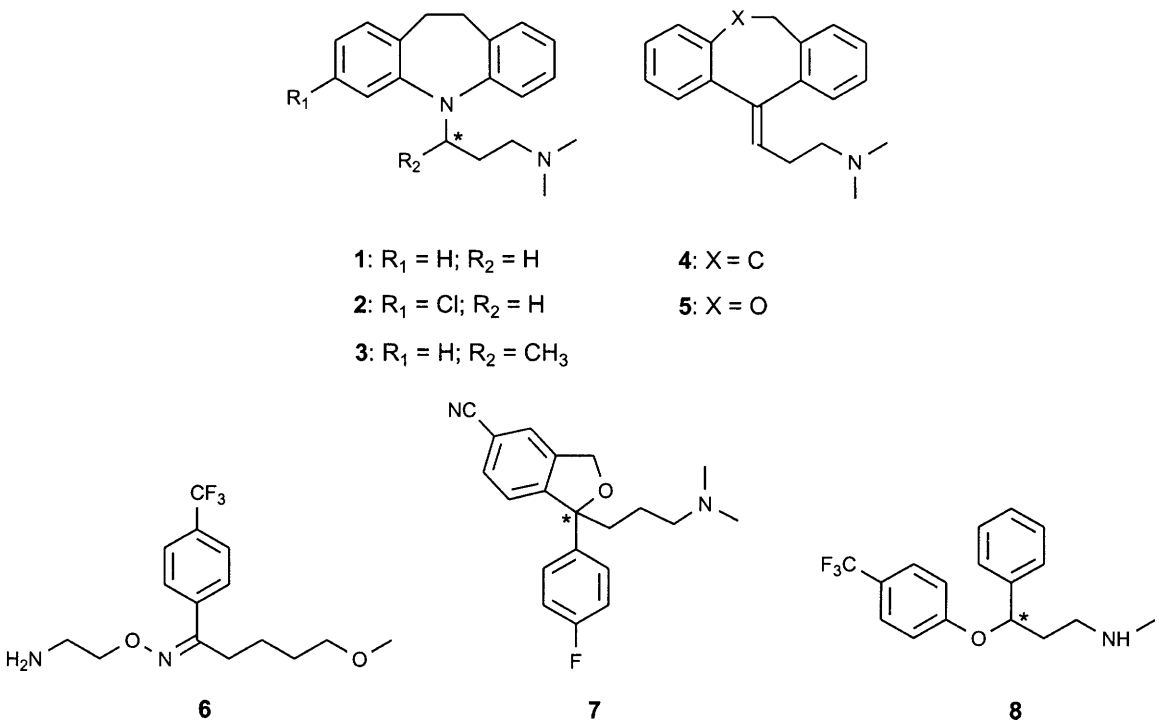

Figure 1 Structures of the investigated compounds: **1**, imipramine; **2**, clomipramine; **3**, trimipramine; **4**, amitriptyline; **5**, doxepine; **6**, fluvoxamine; **7**, citalopram; **8**, fluoxetine. Chiral centres are marked with an asterisk.

where [S] is substrate concentration (Cheng & Prusoff 1973):

$$K_i = IC50/1 + ([S]/K_m) \quad (1)$$

and the Dixon representation. In the case of competitive inhibition and under our conditions ($[S] = K_m$), K_i is IC50/2, and the error on K_i is taken to be half that on IC50.

When the inhibition was of a mixed-competitive type, equations 2 and 3 were used (Oetari et al 1996):

$$K_i = V_{max}{*}K_m[I]/(V_{max}K_m{*} - V_{max}{*}K_m) \quad (2)$$

$$K_i' = V_{max}{*}[I]/(V_{max} - V_{max}{*}) \quad (3)$$

where [I] is inhibition constant, V_{max} is maximal velocity, and where K_m* and V_{max}* were obtained in the presence of inhibitor.

Results

The IC50 and K_i values are presented in Table 1. The IC50 values were only measured for the demethylated metabolites of citalopram and its enantiomers (Table 2). The effect of pre-incubation time on MAO-A and MAO-B inhibition was examined. All compounds seemed to act in a time-independent manner, since inhibition was the same for pre-incubation times of 15 and 5 min.

MAO inhibition by tricyclic compounds

Tricyclic antidepressants displayed approximately 10-times greater inhibitory activity towards MAO-B compared with MAO-A. All showed a mixed type of inhibition towards MAO-A and a competitive type of inhibition towards MAO-B. An example of a Lineweaver-Burk and Dixon plot obtained for MAO-A inhibition by trimipramine is shown in Figure 2.

The K_i values for the competitive MAO-B inhibitors obtained with the Dixon representation and the Cheng-Prusoff equation (Eqn 1) are very similar (Table 1), and their relatively low values reveal a rather high affinity for this isoenzyme.

MAO inhibition by SSRIs

The inhibition of rat brain monoamine oxidases by fluoxetine was studied by Holt & Baker (1996), and has not been re-examined here; only IC50 values were measured revealing a preferential MAO-B inhibitory activity. All SSRIs showed a competitive inhibition mechanism

Table 1 Inhibition of MAO-A and MAO-B by tricyclic antidepressants and selective serotonin reuptake inhibitors.

	MAO-A (μM)			MAO-B (μM)		
	IC50	K_i	K_i'	IC50	K_i[c]	K_i[d]
Imipramine	480 ± 28	380[a]	410[b]	34 ± 2	13	17 ± 1
Trimipramine	540 ± 24	350[a]	950[b]	66 ± 2	32	33 ± 1
Clomipramine	150 ± 12	160[a]	280[b]	45 ± 3	20	23 ± 2
Amitriptyline	430 ± 23	230[a]	1200[b]	38 ± 2	16	19 ± 1
Doxepine	45% (0.75 mM)[e]	ND	ND	98 ± 4	45	49 ± 2
Fluvoxamine	750 ± 43	370[c]	–	400 ± 11	300	200 ± 6
rac-Citalopram	35% (1 mM)[e]	ND	ND	350 ± 12	150	175 ± 6
Fluoxetine	270 ± 18	ND	ND	65 ± 5	ND	ND

[a]Mixed-competitive inhibition, K_i values obtained by Eqn 2; [b]mixed-competitive inhibition, K_i' values obtained by Eqn 3; [c]competitive inhibition, K_i values obtained from Dixon plots; [d]competitive inhibition, K_i values and standard errors obtained from the Cheng-Prusoff equation; [e]inhibition (%) at the concentration indicated (s.d. < 10%; n = 3); ND, not determined.

Table 2 Inhibition of MAO-A and MAO-B by citalopram and its enantiomers and metabolites.

	MAO-A[a]	MAO-B IC50 (μM)
rac-Citalopram	32%	350 ± 12
(*R*)-Citalopram	34%	340 ± 15
(*S*)-Citalopram	34%	360 ± 20
rac-Desmethylcitalopram	34%	400 ± 13
(*R*)-Desmethylcitalopram	40%	340 ± 10
(*S*)-Desmethylcitalopram	40%	460 ± 19
rac-Didesmethylcitalopram	37%	430 ± 26

[a]Inhibition (%) at 1 mM inhibitor (s.d. < 10%; n = 3).

towards MAO-B. Fluvoxamine exhibited a weak MAO inhibitory activity, with a greater potency against MAO-B. Its inhibition mechanism on MAO-A was shown to be competitive. Citalopram and its desmethylated metabolites (Table 2) revealed moderate MAO inhibitory properties, displaying greater activity towards MAO-B. No significant difference was seen between the citalopram derivatives.

Discussion

The activity of the five tricyclic antidepressants towards MAO-A was rather similar, except for clomipramine which exhibited a slightly higher activity than the other drugs. The most pronounced MAO-B inhibitory activity was shown by imipramine and amitriptyline. With only five compounds, no meaningful structure–activity relationship can be derived.

All tricyclic antidepressants tested here showed a mixed competitive inhibition mechanism towards MAO-A and a competitive inhibition towards MAO-B. These results appear to disagree with those reported by Egashira et al (1999), who observed for imipramine a competitive inhibition towards rat brain MAO-A and a non-competitive inhibition towards MAO-B. This discrepancy is believed to be a result of the methods used. Here, MAO-A and MAO-B inhibitory activities were determined with a continuous spectrophotometric assay by monitoring the appearance of the product of the non-selective MAO substrate, kynuramine. Activity against MAO-A and MAO-B was compared after selectively blocking each isoenzyme with deprenyl or clorgyline. In the work of Egashira et al (1999), a radioassay was carried out using serotonin and β-phenylethylamine (PEA) as MAO-A and MAO-B selective substrates. However, although PEA is indeed a better substrate for MAO-B, this selectivity is modest since its K_m value towards rat brain MAO-A is only 3- to 6-times greater than towards MAO-B (Dostert et al 1989). Thus, it does not seem possible to directly compare the results obtained by the two methods. However, there is agreement for selectivity towards MAO-B, despite disagreement on the mechanisms of inhibition.

In the rat, a single dose of amitriptyline (Baumann et al 1984), desipramine (Baumann et al 1983) or fluoxetine (Aspeslet et al 1994) leads to peak brain concentrations in the micromolar range, comparable with their inhibition constants (Table 1). Citalopram is a substrate

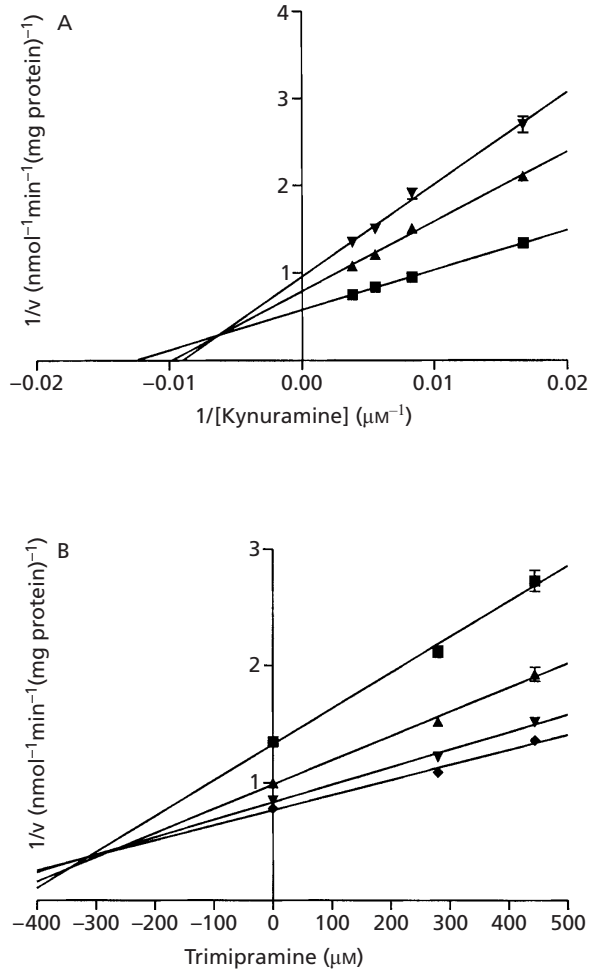

Figure 2 A. Lineweaver-Burk plot of MAO-A inhibition by trimipramine. Homogenates were incubated with trimipramine at 0 μM (■), 280 μM (▲) and 444 μM (▼). B. Dixon plot of MAO-B inhibition by trimipramine. Homogenates were incubated with kynuramine at 60 μM (■), 120 μM (▲), 180 μM (▼) and 270 μM (◆).

of MAO in human liver (Rochat et al 1998), as well as in rat and human brain (Kosel et al 2001); similar studies are not available for other antidepressants. It would therefore be of interest to evaluate the clinical implications of the present findings, especially in situations where MAO inhibitors are combined with tricyclic antidepressants in long-term treatments. As for the co-administration of SSRIs and tricyclic antidepressants, it has been shown that they can produce clinical side-effects and elevated plasma levels of tricyclics (Sokolov & Russell 1995). However, cautious co-administration of tricyclic antidepressants and SSRIs can improve the clinical response and convert non-responders to responders (Eiber & Escande 1999). The capacity of tricyclic antidepressants to inhibit MAO could have clinical implications in such combination therapy. Although it is always difficult to extrapolate in-vitro studies to the clinical reality, the present results suggest further investigations are necessary.

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