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SUICIDE INHIBITION OF MONOAMINE OXIDASES A AND B BY (−)-DEPRENYL

A COMPUTER-AIDED SOLUTION FOR DETERMINING INHIBITION SPECIFICITY

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Abstract—An analytical solution to the differential equations describing the kinetics of the suicide inhibition of a two-enzyme system has been derived and the modelling of suicide inhibition of the monoamine oxidases A and B (MAO A and B, EC 1.4.3.4) by a quasi-selective agent, (−)-deprenyl, is presented as an example. A new parameter, the *specificity index* is defined and used in a model which describes the specific and non-specific binding of (−)-deprenyl to MAO B and MAO A, respectively. This type of analysis may be of therapeutic value by indicating optimal dosage of quasi-selective MAO B inhibitors for the treatment of Parkinson's disease.

N-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP‡) induces Parkinsonian conditions in humans, monkeys and mice [1] by selectively killing nigrostriatal dopaminergic neurones. The mechanism of action involves monoamine oxidase (MAO) B, since MPTP is a selective substrate for this enzyme [2]. MAO B inhibitors prevent the metabolism of MPTP to the effective dopaminergic neurotoxin MPP^+ (*N*-methyl-4-phenyl-1,2-dihydropiridinium ion) [3]. Consequently, selective MAO B inhibitors may have a specific role in the treatment of Parkinson's disease [cf. 4].

(−)-Deprenyl has been found to be a selective inhibitor of MAO B without the "cheese effect" [5] and the value of (−)-deprenyl as an anti-Parkinson agent has been established [cf. 4]. Kinetic studies allowed the inhibitor constants (K_i) for the reversible enzyme–inhibitor complex and the rate constants (k_2) for the step leading to the irreversible transformation of this complex to be determined for the interactions of both MAO A and MAO B with (−)-deprenyl [6–8]. These studies show that the concentration of inhibitor, the relative amounts of the two enzyme forms in particular organs as well as the K_i and k_2 values and the time of exposure of the enzyme to the inhibitor are all important factors influencing the observed "specificity" of the inhibition afforded by compounds such as (−)-deprenyl.

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‡ Abbreviations: MAO A and B, the A and B forms of monoamine oxidase; MPP^+, *N*-methyl-4-phenyl-1,2-dihydropiridinium ion; MPTP, *N*-methyl-4-phenyl-1,2,3,6-tetrahydropiridine; SI, specificity index.

Waley [9, 10] and Tatsunami *et al.* [11] have presented equations describing the kinetics of suicide inhibition for single enzyme systems but, as far as we know, no analysis is available for two-enzyme systems. The inhibitor and substrate specificities of MAO (EC 1.4.3.4) A and B require the development of models describing such a two-enzyme system, since most inhibitors (and substrates) do not show absolute specificity towards only one of the enzymes. Furthermore, selectivity of inhibition does not depend simply on differences between the inhibitor binding affinities of the two forms or on differences between the rates of the irreversible reaction within the non-covalent complex. Both these factors must be taken into consideration (see Fowler *et al.* [7]). To overcome these problems, the *specificity index* is defined and used for interpreting the behavior of such systems. As an example, the parallel suicide inhibition of MAO A and MAO B by (−)-deprenyl is presented since almost all the constants required for computation are known from the literature. Moreover, such an analysis may be of practical value for optimizing drug dosage to provide selectivity of suicide inhibitors acting in bi-enzyme systems.

MATERIALS AND METHODS

Calculations were performed with an IBM PC AT compatible computer using the TK Solver Plus software RK4SA (Universal Technical Systems, Rockford, IL, U.S.A.). This program allows the solution of ordinary differential equations. The procedure is an implementation of the classical 4th order Runge–Kutta method for numerical integration of sets of ordinary differential equations represented

by first-order equations in the form:

$$d(y_i/dt) = f_i(t, y_1, y_2, y_3, \ldots y_n)$$

for $i = 1, 2, \ldots n$.

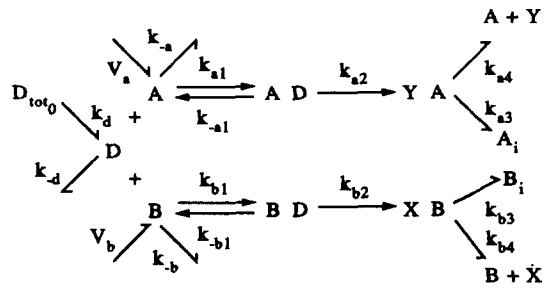
The procedure function RK4SA adjusts the size of the integration steps automatically to maintain a predefined accuracy. Values of constants used in computation are taken from the literature and summarized in Table 1.

(−)-Deprenyl (selegiline hydrochloride; Jumex®) is chemically (−)-*N*-(1-phenylisopropyl)-*N*-methyl-*N*-propinyl-ammonium chloride, a product of the CHINOIN Pharmaceutical and Chemical Works Co. (Budapest, Hungary).

RESULTS AND DISCUSSION

General scheme

The complete kinetic scheme for reversible and irreversible inhibition in a two-enzyme system is outlined in Scheme 1. Uptake ($v_d = k_d[D_{tot}]$) and natural decomposition (k_{-d}) of the inhibitor (D), rates (v_a, v_b) of the *de novo* synthesis of the enzyme forms (MAO A and MAO B, respectively), as well as their degradation (k_{-a}, k_{-b}), e.g. by proteolysis, have been incorporated into the model. The set of differential equations representing the time dependence of the concentrations of the different molecular forms in this system is given in Table 1.



Scheme 1. General scheme of the joint suicide inhibition of a two-enzyme system. Definitions of symbols: A, B, D are the two enzymes (MAO A and B) and the inhibitor [(−)-deprenyl], respectively. D_{tot_0} is the "pool" total concentration of (−)-deprenyl (the actual uptake rate of deprenyl: $v_d = k_d[D_{tot}]$). AD and BD are the *reversible* complexes of MAO A and MAO B with (−)-deprenyl, respectively. YA and XB are the *inactive* covalent complexes of MAO A and MAO B with deprenyl derivatives, respectively. These complexes can be considered as irreversible adducts as their dissociation if any is negligibly slow [cf. 12]. In the general case A_i and B_i are the inactive forms of the two enzymes; however, in the case of inhibition by (−)-deprenyl these forms need not be considered [cf. 12, p. 82]. k_j and k_{-j} are the rate constants of the formation and dissociation (or decomposition) of the different molecular forms, respectively. (The index j = a, b and d means MAO A, MAO B and deprenyl, respectively, while the index 1 corresponds to the reversible complex, index 2 to the inactive complex formation, in the general case index 3 to the inactive enzyme formation and index 4 to the decomposition of YA and XB to active enzymes and transformed inhibitors.)

Table 1. List of differential equations of elementary steps of Scheme 1

$$d[D_{tot}]/dt = -k_d[D_{tot}]$$
$$d[D]/dt = v_d + k_{-a1}[AD] + k_{-b1}[BD] - k_{-d}[D] - k_{a1}[D][A] - k_{b1}[D][B]$$
$$d[A]/dt = v_a + k_{-a1}[AD] + k_{a4}[YA] - k_{-a}[A] - k_{a1}[A][D]$$
$$d[B]/dt = v_b + k_{-b1}[BD] + k_{b4}[XB] - k_{-b}[B] - k_{b1}[B][D]$$
$$d[AD]/dt = k_{a1}[A][D] - (k_{a2} + k_{-a1})[AD]$$
$$d[BD]/dt = k_{b1}[D][B] - (k_{b2} + k_{-b1})[BD]$$
$$d[YA]/dt = k_{a2}[AD] - (k_{a3} + k_{a4})[YA]$$
$$d[XB]/dt = k_{b2}[BD] - (k_{b3} + k_{b4})[XB]$$
$$d[A_i]/dt = k_{a3}[YA]$$
$$d[B_i]/dt = k_{b3}[XB]$$

Definition of selectivity by the specificity index (SI)

The molar ratio of the inactive enzyme species (cf. Scheme 1) is given by $([XB] + [B_i])/([YA] + [A_i])$ in the general case and $[XB]/[YA]$ for the case of MAO and (−)-deprenyl (see later). Variation in this quantity as a function of time is used to characterize the specificity of a suicide inhibitor in a two-enzyme system. This ratio will be termed the *specificity index* (SI). It is not a constant, but a function of several factors, such as the concentration of enzyme forms as well as of the inhibitor and time. Relations between these variables can be described by the set of differential equations listed in Table 1.

Table 2. SI = [XB]/[YA] at $t = 30$ min at different molar ratios of MAO B and A (μM) and at various concentrations of (−)-deprenyl (μM)

[MAO B]	0.01	0.2	0.01	0.04	0.01 [0.1]	0.01	0.0005
[MAO A]	0.0005	0.01	0.0025	0.01	0.01 [0.1]	0.2	0.01
[MAO B]/[MAO A]	20	20	4	4	1	1/20	1/20
Deprenyl							
0.002	1300	1350	260	265	66 [67]	3.3	3.2
0.02	1200	1270	235	240	60 [62]	3.0	3.05
0.2	600	820	115	125	28 [33]	1.5	1.65
2.0	50	90	15	20	3 [4]	0.2	0.3

Other parameters: $v_a = v_b \equiv 10^{-5}$ μM/min $= k_{-a}$; $[A] = k_{-b}[B]$; $k_{a1} = 0.6$ μM^{-1} min^{-1}, $k_{-a1} = 15$ min^{-1}; $k_{b1} = 0.6$ μM^{-1} min^{-1}, $k_{-b1} = 0.6$ min^{-1}; $k_d = 1$ min^{-1}, $k_{-d} = 0.001$ min^{-1}.

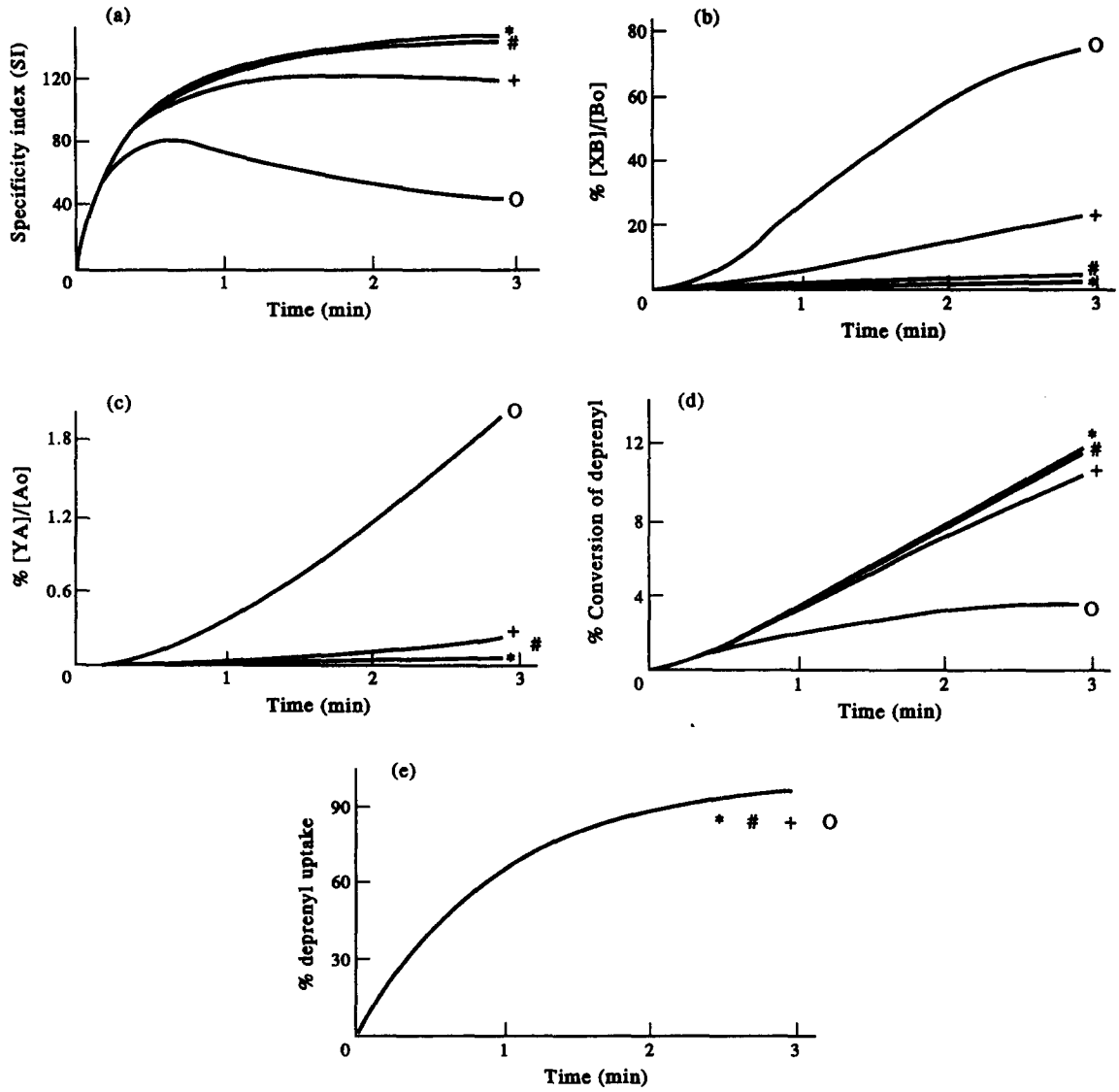


Fig. 1. The effects of time and (−)-deprenyl concentration on the calculated behavior of the model system. All the calculations were carried out according to the system outlined in Scheme 1 and represented by a set of differential equations given in Table 1 with the constants listed in Table 2. Concentration of MAO A = MAO B = 0.1 μM. Symbols *, #, +, O represent deprenyl concentrations 2, 20, 200, 2000 nM, respectively. (a) SI as the function of (−)-deprenyl concentration and time. SI is the ratio of the molar concentrations of XY and YA (see Scheme 1). (b) Percentage conversion of MAO B into its inactive form (XB). The total concentration of MAO B (B_0) in the system is 100%. (c) Percentage conversion of MAO A into its inactive form (YA). The total concentration of MAO A (A_0) in the system is 100%. (d) Percentage conversion of (−)-deprenyl. The total concentration of deprenyl in a compartment where enzymes also exist and can react with it is 100%. This concentration is a function of its uptake rate (v_d; see also Fig. 1e) from the pool containing D_{tot}. (e) Uptake of deprenyl. The total concentration of deprenyl administered in the pool containing D_{tot} at time = 0 is 100%.

Model calculations

The activity ratios of MAO A and MAO B are summarized by Youdim and Finberg [18]. The percentage ratio varies considerably, e.g. in rat brain it is 55:45, in rat liver 50:50, in human brain 20:80, while in human platelet it is 5:95.

Assuming that activity ratios represent the concentration ratios of the two enzymes, calculations with [MAO A]:[MAO B] equal to 1:1, 1:4 and, as extremes, 1:20 and 20:1 have been performed. The results obtained with the 1:1 ratio are shown in Fig. 1, while those for the other ratios are summarized in Table 2. The constants used in these calculations, and the assumptions on which they are based, are given in Table 3.

Figure 1a shows marked differences in SI as a

Table 3. Values of constants used in computation

Remarks	Parameters	Units	Values in Fig. 1
MAO A			
	[A]*	μM	0.1
(1a)	$v_a \equiv k_{-a}$ [A]	μM/min	0.00001
(2a)	k_{-a}	min^{-1}	0.0001
(3a)	k_{a1}	$\mu M^{-1} min^{-1}$	6
	k_{-a1}	min^{-1}	150
(4a)	k_{a2}	min^{-1}	0.14
(5a)	k_{a3}	min^{-1}	0
	k_{a4}	min^{-1}	0
MAO B			
	[B]*	μM	0.1
(1b)	$v_b \equiv k_{-b}$ [B]	μM/min	0.00001
(2b)	k_{-b}	min^{-1}	0.0001
(3b)	k_{b1}	$\mu M^{-1} min^{-1}$	6
	k_{-b1}	min^{-1}	6
(4b)	k_{b2}	min^{-1}	1
(5b)	k_{b3}	min^{-1}	0
	k_{b4}	min^{-1}	0
Deprenyl			
	$[D_{tot}]$	μM	2, 0.2, 0.02, 0.002
(6)	k_d	min^{-1}	1
	k_{-d}	min^{-1}	0.005

The molar ratios (* in pmol/mg protein) of MAO A and B are almost the same (5.5 in rat liver mitochondria and 2.1 in microsomes [13]). Assuming 200 mg/mL total protein concentration [cf. 14] this corresponds to 0.1–0.5 μM of both forms of MAO, which agrees with the value calculated from the kinetic constants (see below).

For the turnover of MAO (1a, b and 2a, b): DellaCorte and Calligham [12] estimated the rate of MAO synthesis by the equation $d[MAO]/dt = k_s - k_d[MAO]$ where the rate of net formation or degradation of the enzyme is balanced between a zero-order rate of synthesis (k_s = 20–30 U/day; with our symbols k_s is v_a and v_b) and a first-order rate constant of degradation (k_d[MAO]) where $k_d = 0.1\ day^{-1}$ (with our symbols k_d—the measure of the rate of degradation by proteolytic enzymes—is k_{-a} and k_{-b}). Using the definition of MAO unit = nmol (mg protein)$^{-1}$.hr^{-1} [cf. 12] values for synthesis and decomposition are about 10^{-5} μM/min and $10^{-4}\ min^{-1}$, respectively. We assumed the same values for both forms of MAO. From these data the steady-state concentration of MAO is equal to $10^{-5}/10^{-4} = 0.1$ μM.

(3a, b) Equilibrium constants $K_{ia} = k_{-a1}/k_{a1} = 25$ μM and $K_{ib} = k_{-b1}/k_{b1} = 0.99$ μM are found in the literature [8] for MAO A and B, respectively, while values for the second-order rate constant of the MAO–substrate complex are given by Gomez *et al.* [13] in the range 6–100 $\mu M^{-1}\ min^{-1}$. On the other hand, second-order rate constants of the formation of enzyme–substrate complexes are in the range of 0.1–100 $\mu M^{-1}\ min^{-1}$ [cf. 15]. We used values of 0.6, 6 and 60 (cf. Table 4) for k_{a1} and k_{b1}, while k_{-a1} and k_{-b1} have been calculated from K_i values.

(4a, b) $k_{a2} = 0.14\ min^{-1}$ and $k_{b2} = 1\ min^{-1}$ have been determined by Fowler *et al.* [7]; however, $k_{b2} = 1\ min^{-1}$ can be considered only as an apparent value, since it depends on the concentration of deprenyl and the enzyme [cf. 7].

(5a, b) Values of k_3 and k_4 for both MAO A and MAO B are close to zero [cf. 16, p. 82] for inhibition by (−)-deprenyl.

(6) The fate of orally administered ^{14}C and ^{3}H double-labelled (−)-deprenyl in different brain segments and in plasma has been studied [17]. From these experiments depletion of radiolabels could have been characterized by a first-order rate constant (k_{-d}) of about $10^{-3}\ min^{-1}$ and $5 \times 10^{-4}\ min^{-1}$ ($T_{1/2}$ = 200 and 1200 min) for ^{14}C and ^{3}H, respectively. Uptake rates (k_d) were found to be too fast to be measurable: at least 100–1000 times faster than depletion.

function of deprenyl concentration and time. SI values indicate how many more molecules of MAO B than MAO A react under the given conditions. Low concentrations of deprenyl (below 200 nM) assure a *quasi*-steady and high (about 120–140 times) selectivity. However, higher concentrations of deprenyl (over 200 nM) and longer times (over 3 min) also result in inhibition of the A form. This is manifested in a significant decrease in SI values with increasing time (cf. Fig. 1a, ○). Consequently, MAO B can be selectively inhibited by a low-dosage administration of deprenyl, but as would be expected, this selectivity is lost at higher concentrations and longer exposure times.

The situation presented in Fig. 1 at time = 3 min and at a deprenyl concentration (200 nM) that is *comparable* with the sum of the MAO B and MAO A concentrations (in Fig. 1 these are each taken as

Table 4. Effect of k_{a1}, k_{b1} on SI at time T_{95}

$k_{a1} \equiv k_{b1}$ μM^{-1} min^{-1}	k_{-a1} min^{-1}	T_{95} min	SI at different deprenyl concns 2 nM	20 nM	200 nM	2 μM	20 μM	200 μM
0.6	15	10	**62**	**61**	48	12	2	Small
6	150	1	**120**	**120**	**112**	68	18	2
60	1500	0.1	**110**	**110**	**110**	**102**	68	22

[MAO A] = [MAO B] = 0.1 *or* 0.01 μM.

* T_{95} is the time in minutes required for the SI to reach about 95% of its maximum at all deprenyl concentrations where SI values are in bold.

$k_{-b1} = 0.6\ min^{-1}$, $k_d = 1\ min^{-1}$, $k_{-d} = 0.001\ min^{-1}$.

0.1 μM) shows that the SI is over 100 (Fig. 1a). About 20% of MAO B but less than 0.2% of MAO A has been inactivated (Fig. 1b and c). Under these conditions the conversion of deprenyl is about 12% (Fig. 1d, +) and its total uptake is nearly 100% (Fig. 1e).

All these data indicate that an optimal dosage of deprenyl would correspond to a concentration comparable with the concentrations of the two MAOs, at least if MAO A and MAO B are present in equal amounts. At higher concentrations (in Fig. 1 this is 2000 nM) selectivity is lost, whereas at lower concentrations (20 nM in Fig. 1) the degree of deprenyl inhibition falls. These effects can thus be summarized as follows: (a) although at higher concentrations MAO A also reacts with deprenyl, as shown by the decreasing SI in Fig. 1a, MAO B is inhibited almost completely within 3 min (cf. Fig. 1b); and (b) with low dosage (2–20 nM) of deprenyl only a very small fraction of MAO B is reversibly inhibited (a few per cent at 3 min; Fig. 1b) which cannot be considered as a significant effect.

The values of the SI have also been calculated at different ratios of the two enzymes. Results at time equal to 30 min are presented in Table 2. These clearly indicate the change of SI at different enzyme ratios and deprenyl concentrations.

The most vulnerable point of the present calculations is the uncertainty in the value of the second-order rate constants (k_{a1}, k_{b1}) for reversible enzyme–inhibitor complex formation since no data are available on them with respect to deprenyl. From different fast reaction measurements, however, Gutfreund [15] gave a range of 0.1–100 $\mu M^{-1}\ min^{-1}$ for enzyme–substrate complex formation in general which fits quite well to values given by Gomez *et al.* [13] for the apparent second-order rate constants of the combination of MAOs with the substrates 5-hydroxytryptamine (0.13 $\mu M^{-1}\ sec^{-1}$ = 8 $\mu M^{-1}\ min^{-1}$) and 2-phenethylamine (2.1 $\mu M^{-1}\ sec^{-1}$ = 120 $\mu M^{-1}\ min^{-1}$).

For this reason we checked the influence of k_{a1} and k_{b1} on SI at different deprenyl concentrations. The results are summarized in Table 4. SI values were calculated over a wide range of deprenyl concentrations (2 nM–200 μM). Values printed in bold characters indicate those deprenyl concentrations where SI is relatively insensitive to concentration changes and where "maximal" selectivity can be obtained at the same reaction time. By increasing k_{a1}, k_{b1} the time required to reach the *quasi*-steady level shortens and the SI increases slightly (60–130).

It can be concluded that the dose of deprenyl *comparable* to the concentrations of the two forms of MAO appears to be optimal for selective irreversible inhibition of MAO B. In order to improve this selective effect, rapid depletion of deprenyl by a side-reaction after time t_{95} (see Table 4) might be useful, preventing its slower undesirable reaction with MAO A. Such an effect may be possible with the administration of a less stable derivative of deprenyl having a k_{-d} value higher than 0.001–0.005 min^{-1}.

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