Antidepressants inhibit human acetylcholinesterase and butyrylcholinesterase activity

Tatiane C. Müller, João Batista T. Rocha, Vera M. Morsch, Roseli Tato Neis, Maria R.C. Schetinger *

Departamento de Química, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, 97105-900, Santa Maria, RS, Brazil

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Abstract

This study examines the effect of the antidepressants fluoxetine, sertraline and amitriptyline on cholinesterase (acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE)) activities in human serum and erythrocyte membrane (ghost). The concentrations used range from 3 to 60 µM for fluoxetine and amitriptyline and 0.3 to 12 µM for sertraline. At the micromolar range concentration, different classes of antidepressants, including fluoxetine and sertraline (selective serotonin reuptake inhibitors (SSRIs)) and amitriptyline (tricyclic antidepressant) inhibited human serum cholinesterase. The order of inhibitory potency was sertraline>amitriptyline>fluoxetine and the IC50 values were 4.05, 9.43 and 62 µM, respectively. Analysis of kinetic data indicated that the inhibition caused by all the antidepressants was mixed in nature. At the micromolar range concentration, sertraline (60–120 µM) and amitriptyline (60–180 µM) inhibited human erythrocyte AChE. The order of inhibitory potency was sertraline>amitriptyline and the IC50 values were 80 and 134 µM, respectively. Analysis of kinetic data indicated that the inhibition caused by all the antidepressants in AChE human erythrocyte membrane (ghost) was mixed in nature. The interaction of sertraline with the cholinesterase is labile since the removal of inhibitor by gel filtration recovered completely the enzyme activity. Our results demonstrate that the usual clinical antidepressants are inhibitors of the cholinesterases on human serum and erythrocyte membrane. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Antidepressant; Cholinesterase; Erythrocyte membrane; Ghost; Serum

1. Introduction

Mammals have two main classes of cholinesterases: acetylcholinesterase (AChE, E.C. 3.1.1.7) and butyrylcholinesterase (BuChE, E.C. 3.1.1.8). These distinct enzymes possess differences in their tissue distribution, kinetic properties, specificity for synthetic and natural substrates and selective inhibitors [1–4] although they are evolutionarily related. For instance, the influence of substrate concentration on enzyme activity is quite different depending on the considered cholinesterase. In fact, AChE is inhibited, while BuChE is activated by an increase in substrate concentration. In humans, AChE is more abundant in the central nervous system, end plate of skeletal muscle and erythrocytes membranes while BuChE is more abundant in serum [5].

BuChE, also called nonspecific cholinesterase or pseudocholinesterase, is able to act on hydrophilic and hydrophobic choline esters [6]. At this moment, the exact physiological function of BuChE is not yet clear, but it is well known that this enzyme hydrolyses a variety of xenobiotics such as aspirin, succinylcholine, heroin and cocaine [7]. Recently, it was suggested that BuChE was found colocalised with senile plaques in the central nervous system, and plays a role in the progressive β-amyloid aggregation and in senile plaques maturation [8].

AChE is an important regulatory enzyme that controls the transmission of nerve impulses across cholinergic synapses by hydrolysing the excitatory transmitter acetylcholine (ACH) [9,10]. Of toxicological and pharmacological significance, AChE is a target for various cholinergic toxins, such as natural snake venom and plant glycoalkaloids, and also a target for therapeutically active compounds, including anti-Alzheimer’s disease drugs. However, there is growing evidence that cholinesterase, probably through their ‘non-cholinergic’ functions, could participate in the pathological
processes in Alzheimer’s disease such as the β-amyloid formation or deposition [11]. In this way, ChE inhibitors may modulate the processing of β-amyloid protein, thus reducing the deposition of β-amyloid itself [12].

The enzyme reaction catalysed by acetylcholinesterase is one of the most efficient reactions known. This efficiency could be due to the three-dimensional structure of the enzyme. The long and narrow active site gorge is about 20 Å deep and includes two sites of ligand interaction: an acylation site at the base of the gorge with the catalytic triad and a peripheral site at its mouth [13]. Some ligands can bind specifically to the acylation or to the peripheral site, and ternary complexes with different ligands bound to each site can be formed.

In BuChE and AChE, the hydrolysis is carried out by a “catalytic triad” of Ser, His and Glu in the active center [14]. Therefore, there are some differences in the catalytic course; for example, BuChE possesses a larger volume in its active site and can be a key factor in determining substrate preference and inhibitor affinity [15].

The clinical antidepressants, fluoxetine, sertraline and amitriptyline are used worldwide [16]. Besides their confirmed efficiency, there are many indirect and undesirable effects presented in the therapy. Trycyclic antidepressants, like amitriptyline, are characterized by a high potential of anticholinergic side effects including memory impairments, delirium, behavioural toxicity and cardiovascular dysfunctions [17]. Although these side effects are considered in the literature, there is no conclusive data about the inhibition of cholinesterases by amitriptyline nor by sertraline and fluoxetine.

Recently, Barcellos et al. [18] reported that acetylcholinesterase from cerebral cortex was inhibited by imipramine, desipramine and amitriptyline at high concentrations. However, the activity of BuChE was not hitherto evaluated with kinetic analysis in the presence of such compounds neither in experimental animals nor in human serum. Furthermore, to the best of our knowledge, the possible anti-cholinesterasic effect of selective serotonin reuptake inhibitors (SSRIs) has not yet been studied in detail.

By the prerogatives outlined above, the purpose of the present investigation was to evaluate whether different antidepressants used in clinical practices induce changes in the cholinesterase activities from human serum and erythrocyte membrane.

2. Materials and methods

2.1. Materials

Acetylthiocholine iodide, DTNB (5,5'-dithiobis(2-nitrobenzoic acid), fluoxetine and imipramine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sertraline was obtained from Galena (Campinas, SP, Brazil). Malathion (technical grade, 95%) was obtained from Indol do Brasil SA (Curitiba, PR, Brazil) and further purified by distillation under vacuum. All other reagents used in the experiments were of analytical grade of the highest purity.

2.2. Humans

Human serum and erythrocyte ghost were obtained from healthy volunteers (18–65 years old from both sexes) of the University Hospital from Santa Maria, with written consent. The protocol was approved by the Human Ethic Committee of the University Hospital from Santa Maria. None of the volunteers had a recurrent or a past history of psychiatric illness, significant medical disorder, or drug, cigarette or alcohol abuse. None of them had been taking any medication for at least the past 30 days.

From all participants, 10 ml of blood was collected in vacutainer tubes, centrifuged at 3000 rpm for 10 min and the serum was collected and used for the enzyme assays.

2.3. Erythrocyte membrane (ghost) preparations

From all participants, 20 ml of blood was collected into heparinized tubes and the membrane was prepared essentially as described by Niggli et al. [19].

2.4. Cholinesterase assay

Cholinesterase activities were determined by the method of Ellman et al. [20], modified by Rocha et al. [21]. Hydrolysis rates v were measured at various acetylthiocholine (S) concentrations (0.008–0.8 mM) in 2 ml assay solutions with 100 mM phosphate buffer, pH 7.5, and 1.0 mM DTNB at 25 °C. One hundred microliters of human serum and 8–12 μg from erythrocyte membrane were added to the reaction mixture and preincubated for 3 min. The hydrolysis was monitored by formation of the thiolate dianion of DTNB at 412 nm for 2–3 min (intervals of 30 s) using a Hitachi 2001 spectrophotometer. All samples were run in duplicate or triplicate. The antidepressants were used in the range of 3–60 μM for fluoxetine, 0.3–12 μM for sertraline and 3–60 μM for amitriptyline. Malathion was diluted in 99% ethanol, to a final concentration of 0.95% in the assay tubes (this final concentration of ethanol did not affect the enzyme activity).

2.5. Protein determination

Protein was assayed by the method of Bradford [22] using bovine serum albumin as standard.

2.6. Kinetic determinations

The kinetic of the interaction of antidepressants and cholinesterase was determined using the Lineweaver–Burk [23] double reciprocal plot, by plotting 1/v against 1/s analysed over a range of acetylthiocholine concentrations.
(0.008–0.8 mM) in the absence and in the presence of fluoxetine (3–60 μM), sertraline (0.3–12 μM) and amitriptyline (3–60 μM). \( K_m \) values were obtained by two different estimates, \( 1/V \) vs. \( 1/S \) [23] and \( V \) vs. \( V/S \) [24–26]. The \( z \) and \( K_i \) values were obtained using the associated plots of \( 1/V \) and \( K_m/V_{max} \) vs. \([I]\). The \( \beta \) value was obtained by the associated plot of \( 1/(K_m)^{o}/(V_{max})^{o} - (K_m)/(V_{max}); \) and \( (1/V_{o} - 1/V_{i}) \) vs. \( 1/[I] \). IC_{50} was calculated according to the Dixon and Webb [27] plot using \( 1/V \) vs. \([I]\) with saturate substrate concentration.

![Chemical structures](image)

Fig. 1. Chemical structures of fluoxetine (a), sertraline (b) and amitriptyline (c).

![Kinetic analysis](image)

Fig. 2. Kinetic analysis of the inhibition of cholinesterase by fluoxetine (a), sertraline (b) amitriptyline (c) in human serum. The graphs show double-reciprocal plots of the cholinesterase experiments in the absence and in the presence of the drugs. Hydrolysis rates \( v \) were measured at various substrate (S) concentrations (0.008–0.8 mM) in 2 ml assay solutions with 100 mM phosphate buffer (pH 7.5) and 1.0 mM DTNB (5,5-di-thiobis(2-nitro-5-phenyl)tetrazolium) using 100 μl of serum and was preincubated for 3 min before substrate addition. All experiments were repeated at least three times with different sources of the enzyme and similar results were obtained. Data presented were from three individual experiments. S.D. values were within 10% of the mean values.
The human serum (500 μl of 99% ethanol solution (to give a final concentration of 0.95% of ethanol) for more 10 min; (B) 10 min with 5 μl of ethanol and sequentially followed by the addition of 5 μl of water was added in the last 10 min; (C) 10 min with 5 μl of sertraline diluted in water (to give a final concentration of 80 μM) in the last 10 min; (D) 5 μl of sertraline diluted in water (to give a final concentration of 80 μM) and after 10 min, the addition of 5 μl of malathion diluted in ethanol (to give a final concentration of 320 μM) and then 5 μl of water was added in the last 10 min; (E) During the first 10 min, serum was preincubated with 5 μl of malathion diluted in ethanol (to give a final concentration of 320 μM) followed by the addition of 5 μl of sertraline (final concentration of 80 μM sertraline) in the last 10 min. Following the second addition, the final concentration of the first drug added was diluted 1%. An aliquot of 100 μl of this preincubated mixture was directly used to determine the enzymatic activity (no filtration), and an aliquot of 200 μl of the preincubated mixture was filtered through a Sephadex 10 (G-10) column. The column was eluted with 10 mM phosphate buffer (1 ml/min) at 25 °C. Finally, 200 μl of the fraction that contained the bulk of protein was considered significant when P<0.05. All analyses were performed using the Statistical Package for Social Sciences (SPSS) software.

### 2.7. Statistical analysis

Data were analysed by one-way and two-way analysis of variance followed by post hoc Duncan’s multiple range test when appropriate. Differences between groups were considered significant when P<0.05. All analyses were performed using the Statistical Package for Social Sciences (SPSS) software.

### 3. Results

At the micromolar range concentration, different classes of antidepressants, including fluoxetine, sertraline and amitriptyline (Fig. 1) inhibited human serum cholinesterase (Fig. 2). The order of inhibitory potency was sertraline>amitriptyline>fluoxetine. Analysis of kinetic data indicated that the inhibition caused by all the antidepressants was mixed in nature. Accordingly, while the $K_m$ values increased, the $V_{max}$ decreased in a concentration-dependent manner. The $K_m$ and $V_{max}$ for serum and erythrocyte ghost AChE were calculated by the double reciprocal method of Lineweaver and Burk [23] and by the method of Eadie and Hofste [24–26] (data not shown). The values of $\alpha$ and $\beta$ and $K_i$ are depicted in Table 1.

The concentration of antidepressants required to inhibit 50% of cholinesterase activity was calculated using the Dixon and Webb [27] plot at appropriate inhibitor concentrations. The $IC_{50}$ values obtained for BuChE inhibition are shown in Table 1.

In order to best characterize the site of interaction between the antidepressant and serum cholinesterase, we examined the influence of sertraline on the inhibitory effect of malathion (Table 2). Malathion is not an inhibitor of cholinesterase; however, commercial preparation of malathion are usually contaminated with malaoxon, which is a very potent cholinesterase inhibitor. In fact, malaoxon binds covalently to a serine located at the bottom of the active site gorge of the enzyme. Data from Table 2 clearly show that malathion (malaoxon) inhibits the cholinesterase in a stable way since the removal of inhibitor by gel filtration in a G-10 column did not restore (reaction B) the original activity. The enzyme activity showed a slight recovery after filtration when compared with enzyme activity that was not filtered in the column. In contrast to malathion, the interaction of sertraline with the cholinesterase is labile since the removal of inhibitor by gel filtration led to complete recovery of the enzyme activity (reaction C). The inhibition of the enzyme caused by the sequential addition of sertraline followed 10 min later by addition of malathion was not different from that observed in the presence of each of these compounds alone (reaction D, no filtration). However, when the enzyme was preincubated first with the antidepressant for 10 min followed by preincubation for 10 min with malathion and then gel filtration, an almost complete recovery of the

### Table 1

<table>
<thead>
<tr>
<th>$\alpha$, $\beta$, $K_i$, and $IC_{50}$ values for cholinesterase from human serum</th>
<th>Fluoxetine</th>
<th>Sertraline</th>
<th>Amitriptyline</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$</td>
<td>2.45</td>
<td>1.81</td>
<td>2.6</td>
</tr>
<tr>
<td>$\beta$</td>
<td>0</td>
<td>0.65</td>
<td>0.16</td>
</tr>
<tr>
<td>$K_i$ (μM)</td>
<td>106</td>
<td>1.08</td>
<td>1.22</td>
</tr>
<tr>
<td>$IC_{50}$ (μM)</td>
<td>32</td>
<td>4.05</td>
<td>9.43</td>
</tr>
</tbody>
</table>

The $\alpha$, $\beta$, $K_i$, and $IC_{50}$ were calculated according Section 2. $\alpha$ is the factor by which $K_m$ changes when the inhibitor occupies the enzyme. $\beta$ is the factor by which $V_{max}$ changes when the inhibitor occupies the enzyme. Values are expressed as mean of three independent experiments performed in triplicate. S.D. values were within 10% of the mean values.

### Table 2

<table>
<thead>
<tr>
<th>Effect of sertraline on malathion-induced cholinesterase inhibition</th>
<th>First 10 min</th>
<th>Last 10 min</th>
<th>Activity (μmol AcSCh/h/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preincubation</td>
<td>No filtration</td>
<td>Filtration</td>
<td></td>
</tr>
<tr>
<td>(A) 5 μl of H$_2$O</td>
<td>5 μl of ethanol</td>
<td>1.30 ± 0.19</td>
<td>1.28 ± 0.14</td>
</tr>
<tr>
<td>(B) 5 μl of malathion in ethanol</td>
<td>5 μl of H$_2$O</td>
<td>0.77 ± 0.10</td>
<td>0.86 ± 0.11</td>
</tr>
<tr>
<td>(C) 5 μl of ethanol</td>
<td>5 μl of sertraline in H$_2$O</td>
<td>0.77 ± 0.10</td>
<td>1.30 ± 0.21</td>
</tr>
<tr>
<td>(D) 5 μl of sertraline in H$_2$O</td>
<td>5 μl of malathion in ethanol</td>
<td>0.73 ± 0.14</td>
<td>1.20 ± 0.11</td>
</tr>
<tr>
<td>(E) 5 μl of malathion in ethanol</td>
<td>5 μl of sertraline in H$_2$O</td>
<td>0.51 ± 0.02</td>
<td>0.73 ± 0.10</td>
</tr>
</tbody>
</table>

The human serum (500 μl) was preincubated for 20 min as follows: (A) 10 min with 5 μl of water and sequentially followed by the addition of 5 μl of 99% ethanol solution (to give a final concentration of 0.95% of ethanol) for more 10 min; (B) 10 min with 5 μl of malathion in 99% ethanol (to give a malathion final concentration of 320 μM) and then 5 μl of water was added in the last 10 min; (C) 10 min with 5 μl of ethanol and then 5 μl of sertraline diluted in water (to give a final concentration of 80 μM) in the last 10 min; (D) 5 μl of sertraline diluted in water (to give a final concentration of 80 μM) and after 10 min, the addition of 5 μl of malathion diluted in ethanol (to give a final concentration of 320 μM); (E) During the first 10 min, serum was preincubated with 5 μl of malathion diluted in ethanol (to give a final concentration of 320 μM) followed by the addition of 5 μl of sertraline (final concentration of 80 μM sertraline) in the last 10 min. Following the second addition, the final concentration of the first drug added was diluted 1%. An aliquot of 100 μl of this preincubated mixture was directly used to determine the enzymatic activity (no filtration), and an aliquot of 200 μl of the preincubated mixture was filtered through a Sephadex 10 (G-10) column. The column was eluted with 10 mM phosphate buffer (1 ml/min) at 25 °C. Finally, 200 μl of the fraction that contained the bulk of protein was used to determine the enzyme activity. The dilution of protein caused by gel filtration varied from 1.8 to 2.3 times. Statistical analysis (two-way ANOVA) with five groups (control, malathion, sertraline, sertraline plus malathion, and malathion plus sertraline) × 2 gel filtration (not filtered and gel filtered) yielded a significant main effect of inhibitors (malathion, sertraline and sertraline + malathion; $P<0.01$) and a significant inhibitors × gel filtration interaction ($P<0.01$).
enzyme activity was observed (reaction D, filtration). In contrast with scheme D, when the serum was first incubated with malathion for 10 min and then exposed to sertraline for 10 min, a stable inhibition of enzyme was observed even after serum was gel-filtered (reaction E, filtration). In the absence of gel filtration, the inhibitory effect caused by preincubation with malathion followed by sertraline was slightly higher than that observed in reaction D (where serum was exposed to sertraline before malathion). In summary, malathion, sertraline and sertraline + malathion inhibited significantly the enzyme activity that was not gel filtered; however, after gel filtration, only the enzyme preincubated with malathion was inhibited. The preincubation with sertraline or sertraline followed by malathion and gel filtration did not result in enzyme inhibition.

At the micromolar range concentration, sertraline and amitriptyline inhibited human erythrocyte membrane (ghost) (Fig. 3). The order of inhibitory potency was sertraline>amitriptyline. Analysis of kinetic data indicated that the inhibition caused by all the antidepressants was mixed in nature. The $K_m$ and $V_{max}$ values obtained for erythrocyte ghost were calculated by the double reciprocal method of Lineweaver and Burk [23] and by the method of Eadie and Hofstee [24–26] (data not shown). The values of $a$ and $b$ and $K_i$ are demonstrated in the Table 3.

### 4. Discussion

The antidepressants tested inhibited cholinesterase activities in an experimental approach using acetylthiocholine as substrate. The inhibitory potency of these antidepressants was different and varied depending on the enzyme source. The differences could be due to chemical variation between these compounds (Fig. 1), which results in distinct interactions with the two classes of cholinesterases and to structural differences between the enzymes tested (BuChE–serum or AChE–erythrocyte membrane), mainly to primary and tertiary enzyme structure peculiarities.

The dimensions and the microenvironment of the active site gorge play a significant role in determining the selectivity, affinity and hydrolysis rate of substrates and inhibitors of esterases [15,28]. In the case of cholinesterase, it is plausible that these compounds bind at the bottom of the active site gorge. Support to this can be obtained in the study of Harel et al. [14], which demonstrated that the crystal structure of tacrine (an inhibitor of AChE with a structure similar to that of sertraline) in AChE binds at the bottom of the active site gorge. The reversible binding of antidepressants to the bottom of the active site gorge may either decrease the velocity of $S$ association to the catalytic site (compatible with a reduction in the forward reaction described below in step 2) or, if the drug remain associated with the enzyme–product complex at the bottom of active site gorge, the drug can cause a decrease in the rate of

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### Table 3

<table>
<thead>
<tr>
<th></th>
<th>Sertraline</th>
<th>Amitriptyline</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a$</td>
<td>1.55</td>
<td>3.17</td>
</tr>
<tr>
<td>$b$</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$K_i$ (µM)</td>
<td>64</td>
<td>143</td>
</tr>
<tr>
<td>IC$_{50}$ (µM)</td>
<td>80</td>
<td>134</td>
</tr>
</tbody>
</table>

$\alpha$, $\beta$, $K_i$ and IC$_{50}$ were calculated according to Section 2. Values are expressed as the mean of three independent experiments performed in triplicate. S.D. values were within 10% of the mean values.
product dissociation from the catalytic site. In this case, the complex \( \text{ChE.Drug.S} \) is converted to \( \text{ChE.Drug.P} \) and the presence of the drug at the bottom of active site makes the dissociation of product (compatible with a decrease in the forward rate on step (5) described below) difficult.

The kinetic analysis of the effects of the antidepressants on cholinesterase activity indicated a mixed inhibition for all compounds because they increased the \( K_m \) value and decreased \( V_{\text{max}} \) value. Further kinetic analysis revealed for fluoxetine that the \( \varphi>1 \) and \( \beta=0 \); these values indicate a mixed inhibition with a competitive partial system and a noncompetitive pure system. Sertraline and amitriptyline presented \( \varphi>1 \) and \( \beta<1 \), indicating a mixed inhibition with a competitive and noncompetitive partial system. The values for \( \varphi>1 \) indicated essentially reversible inhibition.

We propose the following scheme for the interactions between the compounds and cholinesterase:

\[
\begin{align*}
\text{ChE + Drug} & \leftrightarrow \text{ChE.Drug} \\
\text{ChE.Drug + S} & \leftrightarrow \text{ChE.Drug.S} \\
\text{ChE.Drug.S} & \leftrightarrow \text{ChE.S + Drug} \\
\text{ChE.S} & \leftrightarrow \text{ChE.P} \\
\text{ChE.P} & \leftrightarrow \text{ChE + P}
\end{align*}
\]

Both binary (ChE.Drug or ChE.S) and ternary (ChE.Drug.S) complexes could be formed. The complex ChE.Drug.S liberates product at a lower rate, decreasing the \( V_{\text{max}} \). The mixed inhibition type can represent an important experimental characteristic from multisubstrate enzymes, such as cholinesterase.

The results obtained with the column clearly indicated that sertraline hinders the covalent binding of malathion to the esteratic site of the enzyme and experimentally can protect the enzyme from a stable inhibition by malathion. In contrast to malathion, the interaction of sertraline with the cholinesterase is labile since the removal of inhibitor by gel filtration recovered completely the enzyme activity.

Although the enzymes studied in this work measured peripheral cholinergic effects, it can be postulated that the data obtained with erythrocyte membrane enzyme (AChE) can predict some central action of these compounds. This consideration is being postulated since the therapeutic efficacy of the central AChE inhibitors of many compounds, used in clinical routine, is sometimes related to their action on the peripheral AChE from the erythrocyte source. On the other hand, in a similar protocol, it is known that in rat cerebral cortex, imipramine and amitriptyline only inhibit AChE at the millimolar concentration [18]. Therefore, subsequent approaches must be conducted to clarify these data.

In conclusion, it was shown that antidepressants inhibited acetylthiocholine hydrolysis in human serum and erythrocyte membrane by cholinesterases. The concentrations of antidepressants necessary to inhibit serum cholinesterase are relatively close to the clinical levels found after pharmacological use of sertraline and amitriptyline. Consequently, undesirable pharmacological and toxicological side effects of these antidepressants can be related, at least in part, to inhibition of serum and erythrocyte cholinesterases.

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