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Original Article

Inhibition of semicarbazide-sensitive amine oxidase (SSAO) by various antidepressants drugs in monkey platelets

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Summary　I examined whether two or more distinct amine oxidases exist in monkey platelets. Km values of amine oxidase in monkey platelets were determined with benzylamine as substrate from the Lineweaver-Burk double reciprocal plots. Two Km values, 100 and 1.38 μM at high and low benzylamines, respectively, were obtained. When the enzyme in platelets was preincubated at 37 ˚C for 40 min with clorgyline and deprenyl, the deamination of 1 μM benzylamine was not inhibited at high concentrations of these inhibitors, while it was highly sensitive toward semicarbazide. When corresponding experiments were performed with 100 μM benzylamine, opposite results were obtained. Inhibition of semicarbazide-sensitive amine oxidase (SSAO; EC 1.4.3.6) was not influenced by varying the time of incubation of the enzyme with semicarbazide, indicating direct and reversible inhibition of SSAO. These data might suggest the existence of not only monoamine oxidase (MAO; EC 1.4.3.4) but also SSAO in monkey platelets. The mode of inhibition of SSAO was reversible. The most potent inhibition of SSAO was observed with imipramine, followed by maprotiline, zimeldine and nomifensine. The mode of inhibition of SSAO was reversible. Tricyclic antidepressant drugs were most selective inhibitors of SSAO activity in monkey platelets, as compared with other types of antidepressant drugs.

Keywords: Semicarbazide-sensitive amine oxidase (SSAO), Monoamine oxidase (MAO), Antidepressant, Benzylamine, Monkey platelet

Introduction

Semicarbazide-sensitive amine oxidase (SSAO; EC 1.4.3.6) exists in plasma membranes of various tissues and blood plasma[1)]. SSAO differs from monoamine oxidase (MAO; EC 1.4.3.4) and deaminates various xenobiotic amines in mammalian tissues[2)]. SSAO is often characterized by its relatively high activity toward benzylamine, which is also metabolized by MAO. However, the affinity of SSAO toward benzylamine is considerably higher than that of MAO[1)]. Despite its widespread tissue distribution, physiological roles of SSAO remain far from clear[3)]. MAO was classified into two forms, MAO-A and MAO-B, on the basis of their different sensitivities to inhibition by the selective MAO inhibitors, clorgyline and depreny[4)]. In addition, platelet amine oxidase activities in affective disorders such as schizophrenia have been described[5)]. However, there are few studies on possible physiological relationships between platelet amine oxidase activity and psychiatric diagnosis. I have previously reported[6)] that MAO-B in monkey platelets differ from MAO-B in the liver and that it exists in multiple forms. The isoenzymic pattern in diseases[7)] may differ from normal. Therefore, it is considered necessary to investigate the enzymic properties of platelet amine oxidase. I examined whether there might two or more distinct isoenzymes of amine oxidase in monkey platelets. On the other hand, various antidepressant drugs have been developed, and their antidepressive effects have been observed in animal models[8)-10)]. Antidepressant drugs have heretofore been considered effective in the treatment of depression[11),12)]. However, relationships between SSAO and antidepressants are obscure[13)]. Platelet MAO activities in affective diseases such as schizophrenia have been described[14)]. There are few studies on a possible physiological relationship between platelet MAO and

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psychiatric diagnosis[15]. Thus, it is important to investigate enzymic properties of platelet SSAO. Therefore, I examined whether other cyclic or noncyclic antidepressants inhibit monkey platelet SSAO activity.

Experimental

Drugs and chemicals

The MAO inhibitors clorgyline and deprenyl[16] and the amine oxidase inhibitor, semicarbazide hydrochloride, were obtained from Sigma Chemical Co. Ltd., St. Louis, MO., USA. The following drugs used in the study were donated by each manufacture: zimeldine hydrochloride (Fujisawa, Osaka, Japan), imipramine and maprotiline hydrochloride (Chiba-Geigy, Takarazuka, Japan), nomifensine maleate (Hoechst, Frankfurt, Germany). All other chemicals were obtained from Wako Pure Chemical Industries (Osaka, Japan). The radioactive substrate [7-^{14}C]-benzylamine hydrochloride (1.85-2.29 Gbq/mmol) was obtained from Habersham International (Habersham, England).

Monkey platelet preparations

Japanese monkeys (*Malacca furcate*, male 3- to 6-year-old, n=5) were donated by Animal Center, Oita Medical University. This study was approved by the Ethical Committee for Animal Experiments, Oita Medical University, Japan. Blood was quickly removed under chloral hydrate (400 mg/kg i.p.) anesthesia. After sodium citrate anticoagulant (3.8% or 0.12 M) was added to the sample, the blood was centrifuged at 1,100 p.m. for 10 min to remove red cells, and then the supernatant was centrifuged at 3,000 g for 30 min. The pellet containing platelets was suspended in 10 volumes of 0.01 M potassium phosphate buffer (pH7.4) and used as the enzyme preparation. Protein concentrations of the enzyme preparation were measured according to the method of Lowry et al.[17] using the standard of bovine serum albumin.

Assay of SSAO activity

The SSAO activity was measured using the labelled substrates [^{14}C]-benzyl amine, as described previously[18]. The reaction was started by adding the labelled substrate, and the mixture was incubated for 20 min at 25˚C. The reaction was then stopped by adding hydrochloric acid (2N). The products of the reaction were extracted with 2 ml of benzene-ethyl acetate (1:1, V/V) saturated with water. Triton X-100 toluene scintillation liquid (10 ml) was added to 1.0 ml samples of the extract, and the radioactivity was measured in a Beckman LS-9000 scintillation spectrometer. All values are presented as means±S.E.M. In inhibition studies, enzyme preparations were preincubated with various concentrations of semicarbazide for 40 min at 37 ˚C before adding [^{14}C]-benzylamine for the assay of the remaining amine oxidase activity. For investigating the effects of these antidepressant drugs on SSAO activity *in vitro*, the enzyme was preincubated for 20 min at 25 ˚C with antidepressants of concentrations of 1.0 mM from to 1.0 M before adding the substrate. In all cases, product formation was linear with the protein amount and with time periods of incubation used.

Results

The Km values of amine oxidase in monkey platelets with benzylamine used as substrate were determined from the Lineweaver-Burk double reciprocal plots shown in Fig.1.

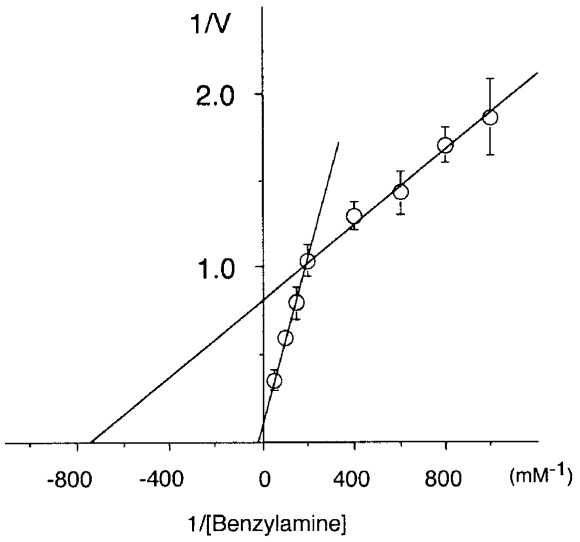

Figure 1

Linewearer-Burk double reciprocal plots of benzylamine deamination by monkey platelets.

Linewear-Burk plots of initial velocity of benzylamine oxidation against benzylamine concentrations were prepared. The enzyme activity was assayed radiochemically by addition of the benzylamine concentration. Two Km values of a high benzylamine concentration (100μM) and a low benzylamine concentration (1.38μM) were obtained. Abscissa: reciprocal of substrate concentration in mM. Ordinate: reciprocal of the initial velocity of the enzyme reaction. Values are means±S.E.M. for five animals.

Two Km values 1.38 and 100 μM, at low and high benzylamine concentrations, respectively. in monkey

platelet were obtained. When the platelets were preincubated at 25˚C for 20 min with clorgyline and deprenyl, the deamination of 1 μM benzylamine by SSAO activity was not inhibited at high concentrations of these inhibitors, while it was inhibited by low concentrations of semicarbazide, the inhibition complete at 10^{-4} M semicarbazide (Fig.2A).

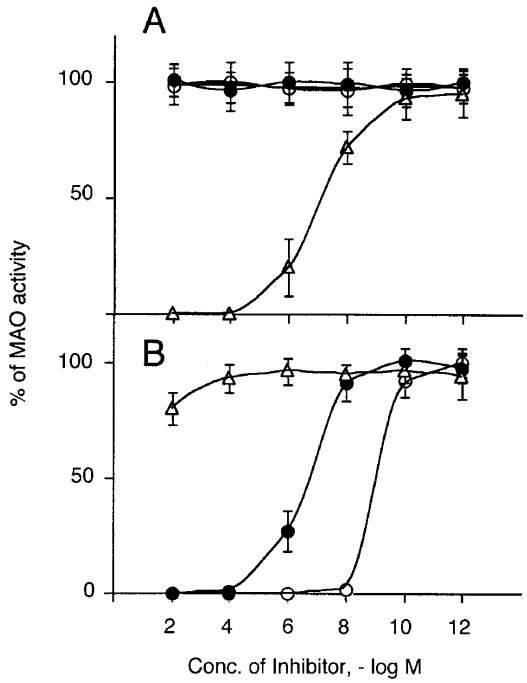

Figure 2

Inhibition of benzylamine deamination in monkey platelets by clorgyline, deprenyl and semicarbazide at 1μM and 100μM benzylamine as substrate.

The enzyme preparation was preincubated with clorgyline (open circle), deprenyl (closed circle) and semicarbazide (triangle). Enzyme activity were assayed radiochemical by addition of 1μM benzylamine at 25 ˚C for 20 min for SSAO activity (A) and 100μM benzylamine at 37 ˚C for 40 min.for MAO activity (B). The remaining activity was expressed as percentage of the control activity. Values are means±S. E.M. for five animals.

Corresponding experiments performed with 100 μM benzylamine at 37 ˚C for 40 min gave opposite results. Deamination of 100 μM benzylamine was highly sensitive toward clorgyline and deprenyl, while it was less sensitive toward semicarbazide as compared with the deamination of of 1 μM benzylamine (Fig.2B). Inhibitory actions of various antidepressant drugs are shown in Fig.3. The time-course of inhibition of SSAO by semicarbazide indicated that the extents of inhibition did not change even for the period of preincubation time up to 60 min at 25 ˚C (Table 1).

This may indicate that semicarbazide is a reversible inhibitor of SSAO in monkey platelets, and that the mode of inhibition is noncompetitive. This enzyme differs from MAO and deaminates various monoamines. All the results presented strongly indicate that the mechanism for inhibition of SSAO by semicarbazide in vitro is certainly different from that of MAO-B. These data might indicate not only the existence of MAO, but also SSAO in monkey platelets. Although the physiological role of SSAO is unknown, the biological significance of this observation is apparent. All four antidepressants inhibit SSAO activity in a concentration-dependent manner. The residual activities of SSAO were about 85.3%, 76.9%, 69.3%, and 16.3% at 100 μM of nomifensine, zimeldzine, maprotililine and imipramine, respectively (Fig.3).

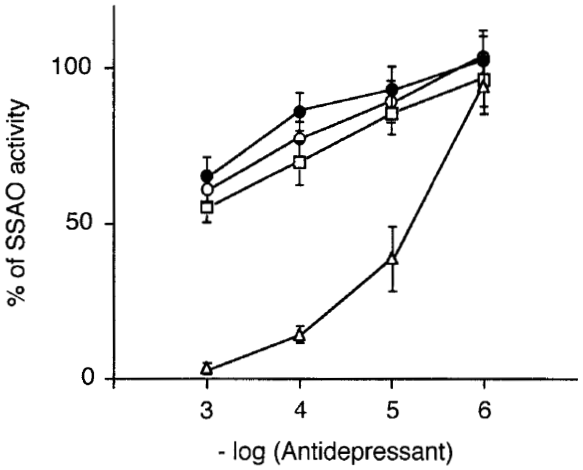

Figure 3

Inhibition of SSAO activity by various antidepressant drugs in monkey platelet.

After incubation at 25 ˚C for 20 min at various concentrations of antidepressant drugs, SSAO activity was determined with 1μM benzylamine as substrate at 37 ˚C for 20 min. Values are expressed as percent of control. The results are means±S.E.M. for six animals; closed circle, nomifensine; open circle, zimeldine, triangle, imipramine; square, maprotiline. Values are means±S.E.M. for six animals.

Discussion

The existence of at least two forms of MAO[2], named A-form MAO and B-form MAO, was first demonstrated by Johston[22]. A-form MAO preferentially deaminates 5-hydroxytryptamine, whereas B-form MAO deaminates β-phenylethylamine[23]. However, benzylamine is the best substrate so far examined for SSAO[1, 2]. It seems likely that total benzylamine deamination at the assay concentration may be mainly due to the difference in the Km values of SSAO (around 5 μM)[20] and MAO (around 160 μM)[21] for this substrate. Consequently, low benzylamine

concentrations can be used for studying benzylamine deamination by SSAO alone that may be present. Discrimination between MAO and SSAO on the basis of substrate concentration was only possible by using 1 μM benzylamine for the assay concentration. In the present work, the data without the MAO inhibitor pretreatment indicated that benzylamine deamination was predominantly by SSAO at the substrate concentration (1 μM) used. In monkey platelets, 1 μM benzylamine was deaminated solely by SSAO. The time-course of inhibition of SSAO by semicarbazide indicated that the degree extents of inhibition did not change even for a preincubation time up to 60 min at 25 ˚C (Table 1).

Table 1

Effect of preincubation time with semicarbazide on semicarbazide-sensitive amine oxidase (SSAO) in monkey platelet.

Preincubation time (min)	Inhibition of SSAO activity (%) caused by semicarbazide at: 0.01 μM	1 μM
0	71.0±4.2	19.3±9.2
20	78.3±5.3	17.6±7.0
40	77.6±6.8	18.9±8.1
60	80.3±7.7	20.3±9.9

Monkey platelets were preincubated with semicarbazide at various concentration at 37 ˚C for the indicated times, and then remaining SSAO activity was assayed, as described in the text. Values were expressed as percent inhibition of SSAO activity with 1μM benzylamine as substrate in preparations preincubated with water for the same periods. Values are means±S.E.M. for five animals.

This may indicate that semicarbazide is a reversible inhibitor of SSAO in monkey platelets, and that the mode of inhibition is noncompetitive. This enzyme differs from MAO in that it and deaminates various monoamines. All results presented strongly indicate that the mechanism for inhibition of SSAO by semicarbazide in vitro is certainly different from that of MAO-B. These data might indicate the existence of not only MAO but also SSAO in monkey platelets. Although the physiological role of SSAO is unknown, the biological significance of this observation may be important.

There are many studies on the relationship between the antidepressant and MAO-inhibiting actions[24)-26)]. However, it is not clear how SSAO inhibition by these antidepressants occurs in platelets. When monkey platelets were used as the enzyme preparation, greatest SSAO inhibition was obtained by tricyclic drug imipramine, followed by tetracyclic maprotiline, dicyclic zimeldine and non-cyclic drug nomifensine. The time-courses of inhibition of SSAO by various antidepressant drugs showed that the extent of inhibition did not change over the preincubation time up to 60 min at 25 ˚C (data not shown), indicating these antidepressant drugs to be reversible SSAO inhibitor in monkey platelets. This may be related to differences in the chemical structures (dicyclic, tricyclic, tetracyclic and noncyclic) [27)] or differences in the binding sites for these antidepressants in the particular animal species used in the present experiments. It is considered that the differences in the sensitivity of monkey platelet SSAO to these antidepressants may exist due to multiple catalytic sites. These antidepressant drugs were found to be selective SSAO inhibitors in monkey platelets. Further studies are necessary to determine whether or not those effects are important with regard to the pharmacologic action of antidepressant drugs. The results of the present study may be useful in elucidating the actual mechanism of the physiological function of SSAO in platelets.

Acknowledgement

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サル血小板中に存在するセミカルバザイドアミン酸化酵素（SSAO）の各種抗うつ剤による阻害

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要 旨

サル血小板中に複数の異なるアミン酸化酵素が存在するか否かについて調べた。ベンジルアミンを基質としてサル血小板中のアミン酸化酵素の Km 値を Lineweaver-Burk 二重逆数プロット法より求めた。それぞれ高（100 μM）と低（1.381μM）濃度の親和性の異なる 2 つの Km 値が得られた。1μM ベンジルアミンを基質として、血小板中の酵素をクロルジリンおよびデプレニルで 37 ℃で 40 分間プレインキュベーションしたところ、高濃度でも阻害されないが、セミカルバザイドでは高い感受性を示した。相当する実験を 100 μM ベンジルアミンを基質として行ったところ、反対の結果が得られた。セミカルバザイドアミン酸化酵素（SSAO）活性はセミカルバザイドでインキュベーションの時間を変えても SSAO 活性は変化しなかったことより可逆的阻害であることが示された。これらのデータよりサル血小板中にはモノアミン酸化酵素（MAO）だけでなく SSAO もまた存在することを示している。その阻害は可逆的であった。SSAO 活性に対してはイミプラミンが最も強く、次にマプロチリン、ジメルジン、ノミフェンシンの順で可逆的であった。三環系抗うつ剤はサル血小板 SSAO 活性に対しては他の抗うつ剤と比べると、最も選択的な阻害剤であった。

キーワード:セミカルバザイドアミン酸化酵素（SSAO）、モノアミン酸化酵素（MAO）、抗うつ薬、ベンジルアミン、サル血小板