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Effects of Higenamine and Its Derivatives on the Activity of Rat Brain Mitochondrial Monoamine Oxidase

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Higenamine과 그 유도체들이 흰쥐 미토콘드리아 Monoamine Oxidase 활성에 미치는 영향

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서유헌 · 박혜영 · 임정규 · 박찬웅

본 연구에서는 higenamine과 그 유도체들이 백서 뇌 미토콘드리아 Monoamine Oxidase(MAO)의 활성에 미치는 영향에 관하여 관찰하였다. 시험한 화합물들 중에서 심장의 등장성 수축에는 효과를 나타내지 않는 methoxyhigenamine이 가장 5-hydroxytryptamine(5-HT)과 phenylethylamine (PEA)에 대한 MAO의 활성을 가역적으로 억제시켰으며, 그 억제 양상은 각각 pure competitive 형과 hyperbolic mixed 형이었다. 이때 5-HT에 대한 IC_{50}는 PEA에 대한 것보다 10배 정도 낮아서 MAO-B 보다는 MAO-A에서 더 강한 억제 작용을 나타내었다. 이로써 methoxyhigenamine은 가역적이며 비교적 MAO-A에 대해 선택적인 MAO 억제제로 사료된다.

INTRODUCTION

In Chinese medicine, Aconiti tuber, the root of Aconiti Radix which belongs to Ranuclaceae plant family, has long been used as a cardiac stimulant, a diuretic or an analgesic. Since Isaku (1958) reported that chloroform insoluble fraction of Aconiti tuber showed inotropic effect on isolated frog heart. Park and his colleague (1981) reported that this effect is potentiated by n-butanol fractionation and the mechanism of inotropic action of Aconiti tuber has been investigated. Recently, Kosuge et al. (1978) isolated from the Aconiti tuber pure cardiotonic compound, higenamine [$C_{16}H_{17}NO_3 \cdot HCl$, dl-1-(4-hydroxybenzyl)-6, 7-dihydroxy-1, 2, 3, 4-tetrahydroisoquinoline HCl] and identified this compound as the active component of Aconiti tuber. Till now, most of the studies on higenamine or n-butanol fraction were largely limited to its cardiac effects. According to the structure of higenamine and its derivatives which related in structure to dopamine, we presumed that they have brain effects, such as effects on monoamine oxidase or dopamine receptor as well as cardiac effects. In the present study we have primarily examined their effects on rat brain mitochondrial monoamine oxidase.

MATERIALS AND METHODS

The rats weighing 200-250g were decapitated and their brains were rapidly removed, weighed and homogenized in 9 volumes of 0.25 M

sucrose-10 mM phosphate buffer, pH 7.4. The pure brain mitochondria were prepared by purifying crude mitochondrial fractions utilizing a discontinuous ficoll density gradient (3% and 6%) procedure by Clark and Nicklas (1970). The mitochondrial fractions were suspended in sucrose buffer and stored at −20°C until use for assay.

MAO activity was determined radiochemically by the method of Flower et al. (1979, 1980) at 37°C and pH 7.4 with incubation for 10 min using ^{14}C-monoamines diluted with unlabeled amines as substrates. In inhibition studies, various concentrations of higenamine or one of its derivatives were added to mitochondrial preparation with or without preincubation for 30 min and the mixture was incubated at 37°C with substrates. Apparent Km values and the mode of inhibition were determined from Lineweaver-Burk plot, with substrates varied over at least a 10 fold concentration range.

Protein concentrations were determined by the method of Lowry et al. (1951) with bovine serum albumin as standard.

The isometric contraction of isolated rabbit heart muscle was recorded by a force displacement transducer connected to a carrier-amplifier as reported previously (Park and Kim, 1981).

Higenamine ($C_{16}H_{17}NO_3 \cdot HI$), higenamine derivative-1 (methoxyhigenamine; $C_{19}H_{23}NO_3 \cdot HCl$), higenamine derivative-2 ($C_{16}H_{15}NO_3 \cdot HI$), higenamine derivative-3 ($C_{19}H_{21}NO_3 \cdot HCl$) and higenamine derivative-4 ($C_{19}H_{21}NO_3 \cdot CH_3$) (Fig. 1) were kindly provided by Dr. H.S. Yoon, Natural Drug Research Institute, Seoul National University, Seoul, Korea. 5-hydroxy-^{14}C-tryptamine binoxalate (5-HT, 50.7 mCi/mmol) and beta-1-^{14}C-phenylethylamine hydrochloride (PEA, 50.0 mCi/mmol) were obtained from New England Nuclear, Boston, Mass.,U.S.A. All other chemicals used were of the highest grade available commercially.

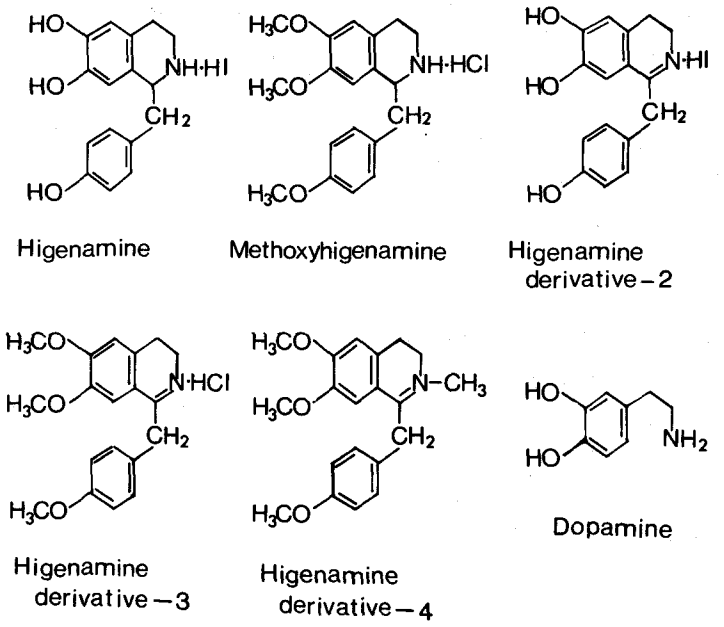

Fig. 1. Structures of higenamine and its derivatives.

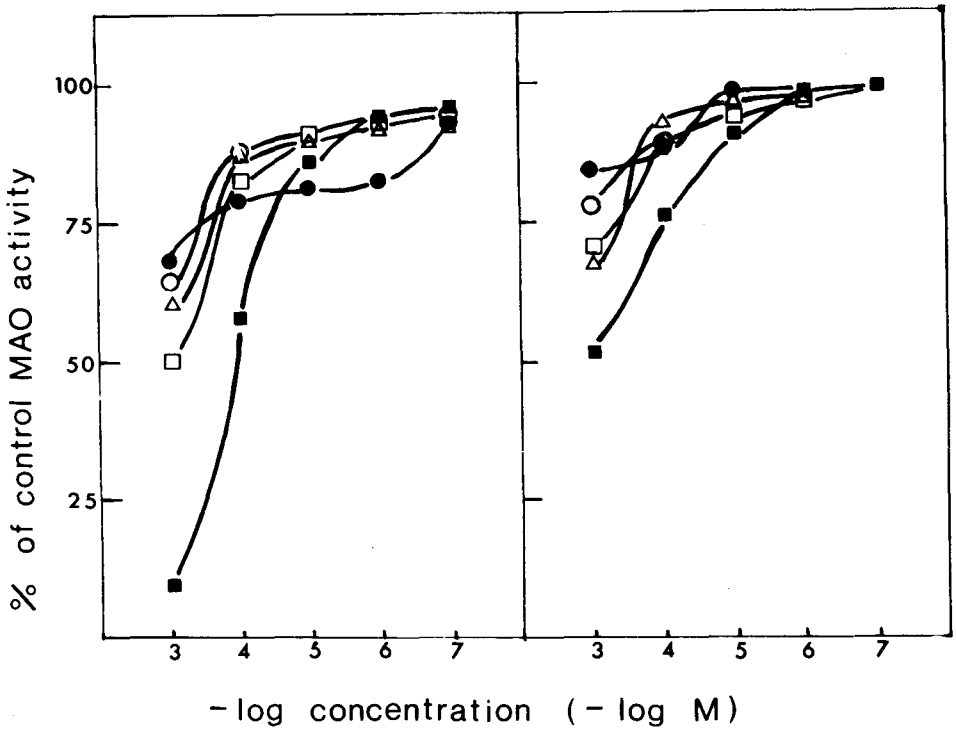

Fig. 2. Susceptability of rat brain mitochondrial MAO activity to higenamine and its derivatives. ●—●: higenamaine; ■—■: methoxyhigenamine; ○—○: higenamine derivative-2; □—□; higenamine derivative-3; Δ—Δ: higenamine derivative-4.
Ordinates: percentage of control MAO activity towards 0.2 mM 5-HT(left) or PEA(right); abscissae: negative logarithm of molar concentration of higenamine and its derivatives. Each point is the mean of duplicate determinations of activity.

RESULTS

a) Inhibition of rat brain MAO by higenamine and its derivatives

The inhibitions of rat brain mitochondrial MAO towards 5-HT and PEA deamination by higenamine and its derivatives are shown in Fig. 2. Of five compounds examined, methoxyhigenamine was the most active MAO inhibitor of them and the IC50 values of methoxyhigenamine to 5-HT or PEA were $1x10^{-4}$M and $1x10^{-3}$ M, respectively. The remainder four compounds demonstrated very mild inhibition of MAO towards 5-HT or PEA. We have examined the effect of methoxyhigenamine on MAO in the following experiments.

b) Inhibition by methoxyhigenamine towards 5-HT deamination

Plotting of the reaction velocity versus amount of enzyme gives an Ackermann-Potter plot which shows whether the inhibitor is reversible or not. When $1.44x10^{-4}$ M methoxyhigenamine was used, the Ackermann-Potter plots were linear and passed through the origin and had a decreased slope compared with uninhibited reaction (Fig. 3). Moreover, same

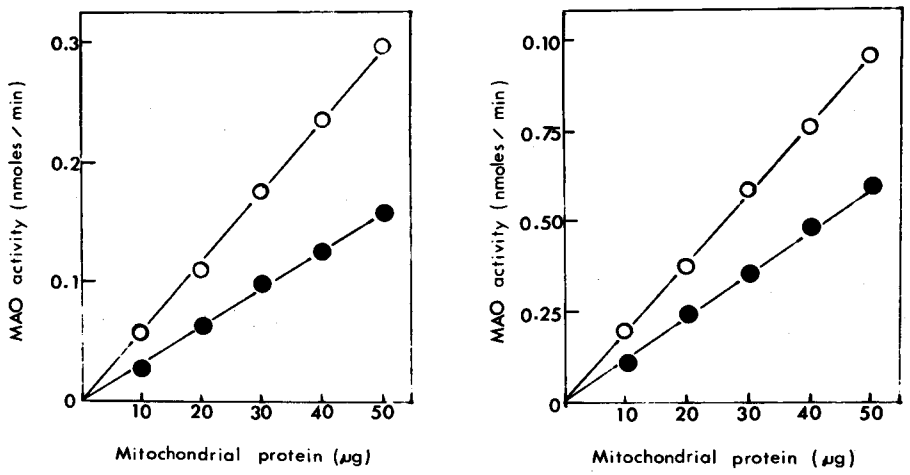

Fig. 3. Ackermann-Potter plot showing the inhibition of MAO towards 5-HT and PEA by methoxyhigenamine. MAO activities were measured by utilizing 0.2 mM 5-HT(left) or PEA(right) as substrates in the absence (○—○) or presence (●—●) of $1.44x10^{-4}$ M(left) or $1.44x10^{-3}$ M(right) methoxyhigenamine. Ordinates:MAO activity towards 0.2 mM 5-HT or PEA(nmoles of 5-HT or PEA metabolized/min); abscissae: amount of mitochondrial protein (μg). Each point is the mean of duplicate determinations of activity in two different preparations.

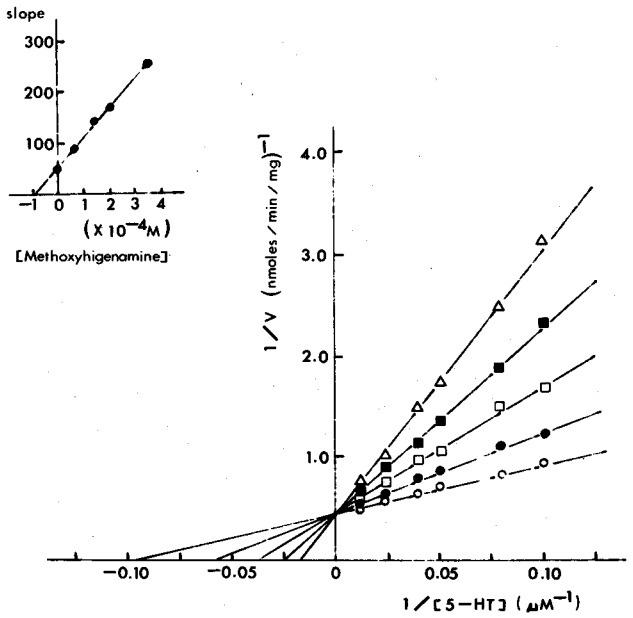

Fig. 4. Lineweaver-Burk plot of the inhibition of MAO towards 5-HT by methoxyhigenamine. Substrate concentration range: 100-800 μM. Samples assayed in the absence (○—○) and presence of $0.72x10^{-4}$ M(●—●), $1.44x10^{-4}$ M(□—□), $2.06x10^{-4}$ M(■—■), $3.60x10^{-4}$ M(Δ—Δ) methoxyhigenamine. Ordinate: 1/initial velocity (nmoles of 5-HT metabolized/min /mg protein)$^{-1}$; abscissa: 1/5-HT concentration(μM^{-1}). Secondary replot is given as inset. The Ki value for methoxyhigenamine towards 5-HT is $8.8x10^{-4}$ M. All values are means of duplicate determinations of activity in four different preparations.

results were obtained after preincubation of methoxyhigenamine and MAO at 37°C for 30 min. This indicates that methoxyhigenamine is a reversible inhibitor of MAO towards 5-HT deamination, regardless of preincubation. As shown in Fig. 4, as the concentration of methoxyhigenamine increased, reduced enzyme activities were accompanied by an increase in the Km values for 5-HT, but by no change in the maximum velocities. The Km value for 5-HT in uninhibited rat brain mitochondrial preparation was 113.3±16.9 μM. In this case, secondary plots of slopes of the graph against the methoxyhigenamine concentrations were linear, and the Ki value of methoxyhigenamine towards 5-HT was 88±8 μM (Fig. 4). These results indicate that the inhibition of MAO by methoxyhigenamine towards 5-HT was shown to be a pure reversible competitive type.

c) Inhibition by methoxyhigenamine towards PEA deamination

When 1.44×10^{-3} M methoxyhigenamine was used, the Ackermann-Potter plots were linear and passed through the origin and had a decreased slope as shown in Fig. 3. The Km value for PEA of uninhibited rat brain MAO was 17.4±0.6 μM. As the concentration of methoxyhigenamine increased, the double reciprocal plots showed upward parallel shift, that was,

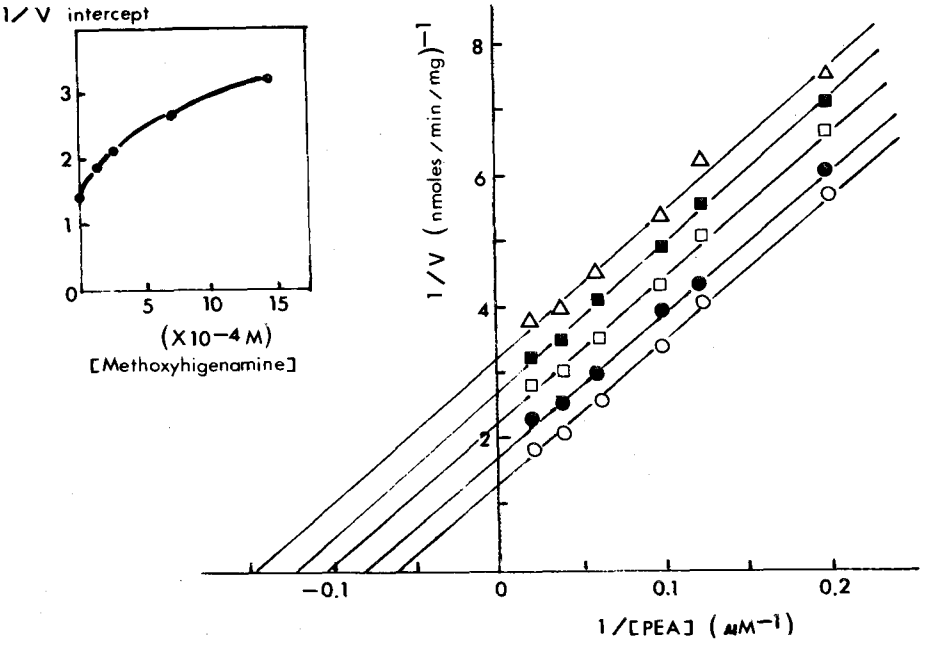

Fig. 5. Lineweaver-Burk plot of the inhibition of MAO towards PEA by methoxyhigenamine. Substrate concentration range: 5-50 μM. Samples assayed in the absence (○—○) and presence of 1.44×10^{-4} M(●—●), 2.88×10^{-4} M(□—□), 7.20×10^{-4} M (■—■), 1.44×10^{-4} M(△—△) methoxyhigenamine. alpha=beta=0.4. Ordinate: 1/initial velocity (nmoles of PEA metabolized/min /mg protein)$^{-1}$; abscissa: 1/PEA concentration (μM^{-1}). Secondary replot or the 1/v-intercepts against inhibitor concentrations is given as inset. All values are means of duplicate determinations of activity in two different preparations.

Table I. Inotropic activities of higenamine and its derivatives in left auricle of rabbit

DRUGS	CONCENTRATION (M)	% OF CONTROL ISOMETRIC CONTRACTION
Higenamine	1.25×10^{-8}	222±5
Derivative-1 (methoxyhigenamine)	1.00×10^{-8}	98±3
Derivative-2	1.00×10^{-8}	103±4
Derivative-3	1.00×10^{-8}	100±2
Derivative-4	1.20×10^{-8}	97±2

Each value represents mean±S.D., n=3, all cases.

Vmax decreased and apparent Km decreased, and alpha and beta were 0.4 in both of them (Fig. 5). Secondary plots of the 1/v-intercepts of the graph against the methoxyhigenamine concentrations were hyperbolic (Fig. 5). These results show that the inhibition by methoxyhigenamine towards PEA was hyperbolic uncompetitive type (hyperbolic mixed type).

d) Effect of higenamine and its derivatives on isometric contraction of rabbit heart muscle

All higenamine derivatives except higenamine did not significantly increase in isometric contraction of rabbit heart (Table 1).

DISCUSSION

Higenamine and its derivatives used in this study, which had dopamine moiety in their structures, inhibited both MAO-A and MAO-B. Methoxyhigenamine was found to be relatively MAO-A selective inhibitor, with IC50 value for 5-HT lower ten fold than for PEA. Methoxyhigenamine reversibly inhibited MAO towards 5-HT and PEA, regardless of the preincubation for 30 min. This differs from known irreversible MAO inhibitors such as pargyline, clorgyline and deprenyl which bind covalently and irreversibly to a flavin moiety of MAO after a reversible phase of inhibition as preincubation time goes on (Hellerman and Erwin, 1968; Johnston, 1968; Knoll and Magyar, 1972; Lyles and Greenawalt, 1977; Oreland and Ekstedt, 1972; Oreland et al., 1973). Moreover, methoxyhigenamine inhibited MAO towards 5-HT and PEA, in a pure competitive fashion and in a hyperbolic uncompetitive (mixed) fashion, respectively without effect on isometric contraction of heart.

The existence of two major species of MAO that have different substrate specificities and inhibitor sensitivities allows the possibility of using specific inhibitors to achieve specific pharmacologic effects. Selective inhibition of MAO-A causes elevated neuronal concentrations of the transmitter amines 5-hydroxytryptamine and noradrenaline resulting in greater amounts of the transmitter being released upon impulse activity. Selective MAO-A inhibitors are accordingly therapeutically interesting as effective anti-depressant agents. There are several drawbacks with most of the existing MAO inhibitors. They are non-selective and irreversible inhibitors and they strongly potentiate the cardiovascular effects of tyramine and

similar sympathomimetic amines.

In conclusion, methoxyhigenamine, which has no effect on isometric contraction of heart, is a reversible, relatively MAO-A selective inhibitor in vitro and further work is required to determine in vivo effects of methoxyhigenamine.

= Abstract =

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The effect of higenamine and its derivatives on the activity of rat brain mitochondrial monoamine oxidase(MAO) was studied. Methoxyhigenamine of drugs tested had no effect on isometric contraction of heart and reversibly inhibited MAO towards 5-hydroxytryptamine (5-HT) and phenylethylamine(PEA) in a pure competitive fashion and in a hyperbolic mixed fashion, respectively, but was found to be relatively MAO-A selective inhibitor, with IC50 value for 5-HT lower ten fold than for PEA. The results suggest that methoxyhigenamine is a reversible, relatively MAO-A specific inhibitor in vitro.

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