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수종 모노테르펜계 화합물이 랫드 뇌의 monoamine oxidase활성에 미치는 영향

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Effects of Some Monoterpenes on Rat Brain Monoamine Oxidase

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ABSTRACT— Eight natural or semisynthesized monoterpenes were examined for their effects on rat brain monoamine oxidase(MAO) using benzylamine as substrate. Thujone and 3-carene were found to have the inhibition effects on rat brain MAO activity; 38% and 95% inhibition at 10^{-3} M respectively. The kinetic study on 3-carene, the most potent inhibitor tested in this study, showed that its MAO inhibition effect was confirmed as uncompetetive type. But (+) pulegon and (-) isopulegon was found to activate MAO slightly.

Key words □ brain monoamine oxidase, monoterpenes, 3-carene, kinetic study

Several monoterpenes have been found to have central nervous system (CNS) stimulating effect and some monoterpenoids such as thujone have been suggested to have psychoactivity similar to cannabinoids. It was also reported that thujone and tetrahydrocannabinol(THC) exert their psychotomimetic effects by interacting with a common receptor in the CNS[1] and thujone showed mild hallucination effect similar to the marijuana, the psychoactive component of which, THC, was reported to inhibit porcine brain mitochondrial MAO activity.[2] Therefore we supposed that this hallucination effect might be due to the accumulation of biogenic amines in brain.

As a basic study to elucidate the possible interrelationship between CNS-effects of monoterpenes and their MAO-inhibition effects, we investigated the effects of some natural or semisynthesized monoterpenes on the activities of MAO from rat brain.

MATERIALS AND METHODS

Reagents and animals

Eight monoterpenes used in this study were obtained from one of authors, Dr. C. K. Ryu. Benzylamine (E. Merk) was recrystallized and used as HCl salt form. Benzaldehyde (Junsei Chem) was refined according to its conventional refining method and used as U.V. spectrophotometric standard. All other chemicals used were guaranteed reagent grade. Wistar rats, weighed 150~200 g, were obtained from the Experimental Animal Farm of Seoul National University.

Preparation of enzyme suspension

Wistar rats, weighed 150~200 g, were killed by decapitation and their whole brains were removed immediately and cooled in small volumes of 0.32 M ice-cold sucrose solution. It was once washed and weighed. 10% homogenate (w/v) was prepared in 0.32 M sucrose solution using Potter-Elvehjen homogenizer with teflon pestle.

The brain homogenate was centrifuged at 1,000×g for 10 minutes to remove nuclei and cell debris. The pellet was washed once with 1 m*l* of 0.32 M sucrose solution. The supernatants and washing were recentrifuged at 12,000×g for 20 minutes to yield the crude mitochondrial fraction and the supernatants were discarded. The pellet was washed once with small volumes of same solution and washings

were discarded. After the pellets were suspended in 3 m*l* of same sucrose solution by agitation with vortex mixer, these crude mitochondrial suspensions were stored at -20 °C until use for assay.

Protein was determined by the method of Lowry *et al.* using bovine serum albumin as standard.[3] Diluted solutions (25~200 r protein) of bovine serum albumin were used as resulted working standards.

Crude mitochondrial suspension was diluted 200 fold and the amount of protein was calculated from standard curve.

Determination of MAO activity[4]

About 0.5~1.0 mg of protein of rat brain mitochondrial fraction was incubated in 0.05 M tris-HCl buffer, pH 8.2, containing 3 mM benzylamine HCl at 37 °C for 30 minutes.

At the end of incubation the reaction test tube was immediately cooled in ice-bath and 1 m*l* of 3% ice-cold zinc sulfate solution was added and mixed thoroughly with vortex mixer. The precipitate was easily settled down within 30 minutes in ice-bath. After centrifugation of this cooled reaction mixture at 3,000 rpm, for 5 minutes, the absorbance of the clear supernatant was measured at 250 nm spectrophotometrically.

The blank was prepared with zinc sulfate-pretreated (inactive) enzyme preparation. All operations were carried out on triplicates and the results were expressed as amount of benzaldehyde produced/mg protein

RESULTS AND DISCUSSION

It is generally known that depression is caused by diminished activity of the norepinephrine and serotonin systems. Depressive patients can be treated very effectively with the drugs that increase norepinephrine and serotonin at the nerve endings such as monoamine oxidase (MAO) inhibitors. From this reason many natural or synthetic compounds have been investigated on their effects on MAO activity.[5-8]

In this study, eight natural or semisythetic monoterpenes were examined for their effects on the rat brain monoamine oxidase activity. The activity of MAO towards benzylamine was assayed by the spectrophotometric method of Tabor *et al.*[9]

The MAO activity was influenced by the change of pH and the kind of buffer used; slight increase in MAO activity was observed by increasing pH. Extinction coefficient of bezyladehyde was known as 13.8×10^3 $ml^{-1}cm^{-1}$, but the value obtained from this experiment was slightly lower. In order to establish the standard assay condition, proper ranges of the incubation time and substrate concentration were investigated and the linearity was proved when 0.5~1.0 mg of protein of the mitochondrial fraction was incubated with benzylamine for 30 minutes at the assay condition of pH 8.2, 37 °C.

Under this condition, the velocity of the enzyme reaction was linear for 30 minutes and was proportional to the activity of the monoamine oxidase. 3 mM of benzylamine was found to be a substrate saturated concentration and no inhibition by the excessive substrate was observed at this concentration. Km value was determined as 53 μM from Lineweaver and Burk plot.

As the substances tested are not soluble in aqueous media, it was necessary to find a proper solvent to solubilize them. Alcohols are generally good solvents for these essential oils, but the effects of them on monamine oxidase have not yet clearly elucidated. Jurosawa has reported that 0.1 to 10% methanol and ethanol increased monoamine oxidase activity in both beef and rat liver mitochondria.[10]

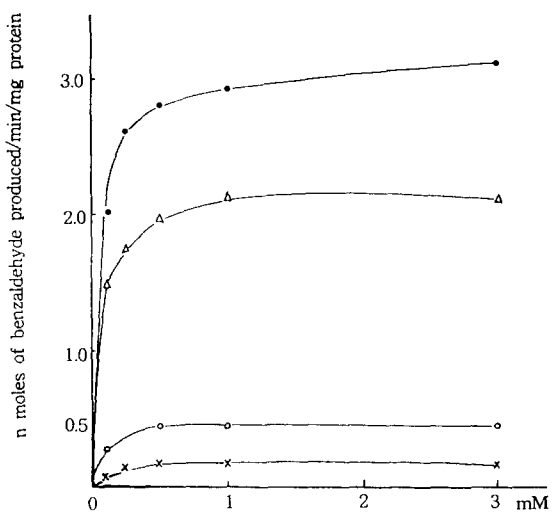

Fig. 1. Inhibition of Rat Brain MAO by 3-Carene.
●–● Control, △–△ 10^{-6} M,
○–○ 10^{-5} M, ×–× 10^{-4} M

Table 1. Percent Representation of the Effects of Some Monoterpenes on Rat Brain MAO Activities

Structure	Name	Activity(%)		
		10^{-3}	10^{-4}	10^{-5}
	Thujone	38.3	62.0	85.0
	3-Carene	3.3	5.4	12.2
	4-Isocaranone	100.0	103.9	101.3
	3-Hydroxymethyl-Caran-4-on	108.3	105.7	102.6
	(+) Carvon	91.2	99.0	101.0
	(-) Carvon	100.9	102.1	102.1
	(+) Pulegon	150.1	143.0	114.5
	(-) Isopulegol	112.5	114.5	108.6

*MAO activity is expressed as the percentage of the control.

From these points of view, we tried to search a suitable solvent, propylene glycol was found to be a proper solvent, which had no effects on monoamine oxidase activity. Inhibition studies were carried out at the concentrations of propylene glycol less than 1.5%.

The results from the experiments investigating the effects of monoterpenes on the monoamine oxidase activities are shown in Table 1. Among them 3-carene and thujone were found to be potent monoamine oxidase inhibitors: 95% and 38% inhibition at 10^{-4} M respectively.

3-carene was confirmed as most potent MAO inhibitor among the eight natural or semisynthetic terpenes tested in this study. Kinetic study on the MAO-inhibition effects of 3-carene showed that the inhibition effect was identified as an uncompetetive type.

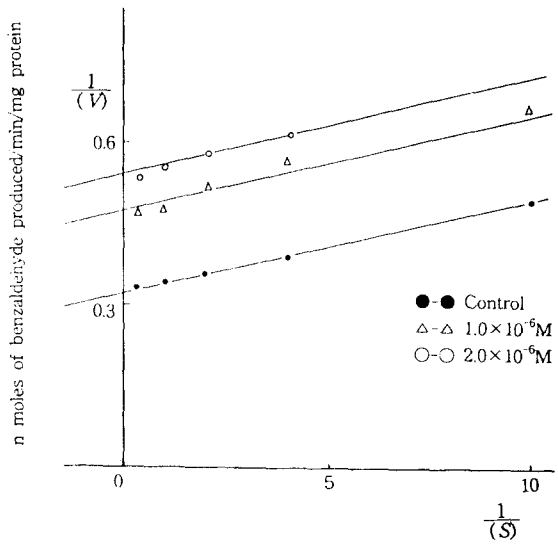

Fig. 2. Lineweaver and Burk Plot of Inhibition of Deamination of Benzylamine by the Treatment of 3-Carene.

As shown in Fig. 2, Ki value for benzylamine was determined. But, it should be clarified whether its inhibition type is based on the action of Tris buffer or not. Interrelationship of the psychotomimetic action of thujone derivatives with their MAO inhibition effect remains also to be elucidated.

4-Isocaranone, 3-Hydroxymethyl-Caran-4-4-on, (+) Carvon, (-) Carvon, (+) Pulegon and (-) Isopulegol did not show any significant effect on MAO activity. But (+) pulegon and (-) isopulegol showed rather slight activating effects on MAO activities.

국문요약

일부 모노테르펜계 화합물은 중추신경계를 자극하는 효과가 있는 것으로 알려져있다. 본 연구에서는 모노테르펜계 화합물의 중추신경계에 대한 효과가 monoamine oxidase의 활성을 저해함으로써 이루어지는 지를 확인하기 위한 기초연구로써 8종의 모노테르펜계 화합물이 랫드 뇌의 monoamine oxidase에 미치는 영향을 살펴보았다. Thuione과 3-carene은 10^{-3} M의 농도에서 랫드 뇌의 MAO활성을 각각 38%, 95% 정도로 억제하는 것으로 밝혀졌다. 그러나 (+) pulegon and (-) isopulegon 은 약간 활성을 증가시키는 것으로 나타났다. 동력학적 실험 결과에 의하면 3-carene의 MAO저해 활성은 비경쟁적인 것으로 확인되었다.

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