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INHIBITION OF TYPE A MONOAMINE OXIDASE BY 2(*N*)-METHYL-6,7-DIHYDROXYISOQUINOLINIUM IONS

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Abstract—In the human brain, monoamine-derived 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinolines and 1,2,3,4-tetrahydroisoquinolines have been identified and their enzymatic methylation into *N*(2)-methylisoquinolines has been also confirmed. *N*-methylated 6,7-dihydroxyisoquinolines were found to be oxidized into 6,7-dihydroxy-*N*-methylisoquinolinium ions. The effects of the isoquinolinium ions on type A and B monoamine oxidase were examined, using enzyme samples isolated from human brain synaptosomal mitochondria. 1,2-Dimethyl-6,7-dihydroxyisoquinolinium ion (*N*-methylsalsolinium ion) and 2-methyl-6,7-dihydroxyisoquinolinium ion (*N*-methylnorsalsolinium ion), were found to be potent inhibitors of type A monoamine oxidase. The inhibition was competitive to the substrate, while the isoquinolinium ions were much weaker inhibitors of type B and the inhibition was non-competitive to the substrate. Isoquinolinium ions without catechol structure, *N*(2)-methylisoquinolinium ion and 1,2-dimethylisoquinolinium ion also inhibited both type A and B monoamine oxidase. 1,2-Dimethylisoquinolinium was the most potent inhibitor among examined isoquinolines, followed by the *N*-methylsalsolinium ion. The activity–structure relationship of the isoquinolines with and without catechol structure was examined in terms of potency and selectivity of the inhibition to type A and B monoamine oxidase. Catechol structure was found to increase the selectivity of inhibition to type A, as shown by comparison of *N*-methylsalsolinium ion with 1,2-dimethylisoquinolinium ion. *N*-Methylsalsolinium ion inhibited type A MAO more selectively than 1,2-dimethylisoquinolinium ion, which inhibited type A and type B with almost the same values of the inhibitor constant. The selective inhibition of type A monoamine oxidase by catechol isoquinolinium ions may have an important role in the brain function, since the substrates of type A monoamine oxidase are major neurotransmitters in the brain, such as serotonin and norepinephrine. The inhibition of monoamine oxidase by isoquinolinium ions may perturb the levels and function of the monoamines in the brain under physiological and pathological conditions.

Monoamine oxidase [monoamine: oxygen oxidoreductase (deaminating), EC 1.4.3.4, MAO] is the major catabolic enzyme of catecholamines and indoleamines in the brain. It is classified into type A and B, according to its sensitivity to the specific inhibitors and its substrate specificity (Johnston, 1968). Serotonin (5-hydroxytryptamine) and norepinephrine are the substrates of type A monoamine oxidase (MAO-A) and dopamine is the substrate of both type A and B. β-Phenylethylamine and benzylamine are the substrates of type B (MAO-B). It is now established that these two types are composed of genetically different protein molecules (Bach *et al.*, 1988). MAO activity is one of the major factors regulating the monoamine levels in the brain and inhibitors of MAO, such as deprenyl, an irreversible MAO-B inhibitor, are now applied for treatment of some neurological diseases or psychiatric diseases, such as Parkinson's

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Abbreviations. MAO, monoamine oxidase; MAO-A and -B, type A and B monoamine oxidase; Sal, salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline); Norsal, norsalsolinol (6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline); TIQ, 1,2,3,4-tetrahydoisoquinoline, NMTIQ, *N*(2)-methyl-1,2,3,4-tetrahydroisoquinoline; $NMIQ^+$, *N*-methylisoquinolinium ion; NMSal, *N*(2)-methyl salsolinol; NMNorsal, *N*-methylnorsalsolinol; $NMSal^+$, *N*-methylsalsolinium ion, $NMNorsal^+$, *N*-methylnorsalsolinium ion; $1,2\text{-DiMeIQ}^+$, 1,2-dimethylisoquinolinium ion; 1-MeTIQ, 1-methyl-1,2,3,4-tetrahydroisoquinoline.

disease (Birkmayer *et al*., 1977) and depression (Mann and Gershon, 1980). On the other hand, data have been accumulated to indicate that there are many naturally-occurring MAO inhibitors in the brain. isoquinoline alkaloids (Bembenek *et al*., 1990) and quinolines and quinaldines (Naoi and Nagatsu, 1986, 1988). One of these isoquinolines, 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (salsolinol, Sal) was detected for the first time in the urine of the patients administrated with L-DOPA (Sandler *et al*., 1973) and then also in the human brain (Sjoequist *et al*., 1982). Sal is synthesized in the brain by non-enzymatic condensation of dopamine with pyruvic acid, followed by decarboxylation and reduction, which gives rise (*R*) enantiomer (Strolin-Benedetti *et al*., 1989, Dostert *et al*., 1990). Another tetrahydroisoquinoline, 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (norsalsolinol, Norsal) was also detected in rat brain (Barker *et al*., 1981). An isoquinoline without catechol structure, 1,2,3,4-tetrahydroisoquinoline (TIQ), occurs in the human brain (Niwa *et al*., 1987) and was reported to elicit parkinsonism in monkeys (Nagatsu and Yoshida, 1988). *N*(2)-Methylation of this isoquinoline into *N*-methyl-1,2,3,4-tetrahydroisoquinoline (NMTIQ) was confirmed in the brain (Naoi *et al*., 1989a) and the *N*-methylated isoquinoline was oxidized into *N*-methylisoquinolinium ion ($NMIQ^+$) (Naoi *et al*., 1989b). Recently we found that 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinolines also are methylated at the *N*(2) position. *N*-methylation of (*R*)Sal into 1,2-dimethyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (NMSal) was confirmed by *in vivo* microdialysis of the rat brain (Maruyama *et al*., 1992). NMSal and *N*-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (*N*-methylnorsalsolinol, NMNorsal) were identified in the human brain by gas chromatography-mass spectrometry (Niwa *et al*., 1991). More recently, oxidation of NMSal and NMNorsal into *N*-methylsalsolinium ion ($NMSal^+$) and *N*-methylnorsalsolinium ion ($NMNorsal^+$) were confirmed (Naoi *et al*., 1994). These results indicate that in the brain there are many endogenous isoquinolines, which may inhibit MAO activity and perturb the *in vivo* metabolism of monoamine neurotransmitters. In fact, the inhibition of type A monoamine oxidase by the reduced forms of salsolinols was confirmed (McCrodden *et al*., 1988, Bembenek *et al*., 1990). Using human synaptosomal mitochondria as MAO samples, inhibition of MAO-A and MAO-B activity by Norsal and NMNorsal was reported. The induction of a methyl group into the *N*(C2) position proved to increase the affinity to MAO (Minami *et al*., 1993). Previously, the oxidative product of NMTIQ, $NMIQ^+$, was found to inhibit MAO-A more potently than TIQ (Naoi *et al*., 1987, 1989c) which suggests that other isoquinolinium ions with or without catechol structure may be potent inhibitors of MAO.

In this paper, the effects of *N*-methylisoquinolinium ions, with or without catechol structure, on MAO activity were systematically studied using the enzyme samples prepared from human brain synaptosomal mitochondria. The effects of the chemical structure of isoquinolines on the selectivity and potency of inhibition of type A and B MAO were examined among *N*-methylisoquinolinium ions and compared with tetrahydroisoquinolines, *N*-methyl-1,2,3,4-tetrahydroisoquinolines with or without catechol structure. Possible involvement of MAO inhibition by the naturally-occurring isoquinolines to the levels and function of the brain monoamines is discussed.

EXPERIMENTAL PROCEDURES

Assay for MAO activity

Synaptosomes were prepared from human brain cortex (frontal lobe), according to the method by Gray and Whittaker (1962). MAO activity was measured fluorimetrically using kynuramine as a substrate by Kraml's method (1965). To differentiate type A and B activity, the MAO samples were preincubated at 37°C for 10 min with 1 μM deprenyl or clorgyline, respectively. Kinetics of the type A and B MAO were studied with 8 different concentrations of the substrate with 100 or 10 μM isoquinolines. The type of inhibition and the value of the inhibitor constant, K_i, were determined by plotting the data according to Lineweaver and Burk. The protein concentration was measured according to Bradford (1976), using bovine γ-globulin as standard.

Isoquinolines and other chemicals

Salsolinol, and their *N*-methylated compounds were synthesized according to Teitel *et al*. (1972). 1-Methyl-1,2,3,4-tetrahydroisoquinoline (1-MeTIQ) and 1,2-dimethylisoquinolinium ion (1,2-DiMeIQ$^+$) were prepared as reported previously (Barrows and Lindwall, 1942). Kynuramine was purchased from Sigma. Clorgyline and deprenyl were kindly donated by May and Baker and Dr Knoll, Department of Pharmacology, Semmelweis University (Budapest, Hungary), respectively.

RESULTS

Figure 1 shows the chemical structures of *N*-methylisoquinolinium ions with and without catechol structure and their metabolic precursor isoquinolines used in these experiments.

The K_m and V_{max} values of MAO-A and B samples are presented in Tables 1 and 2. In the enzyme sample, MAO-B activity was much higher than type A, 2.67

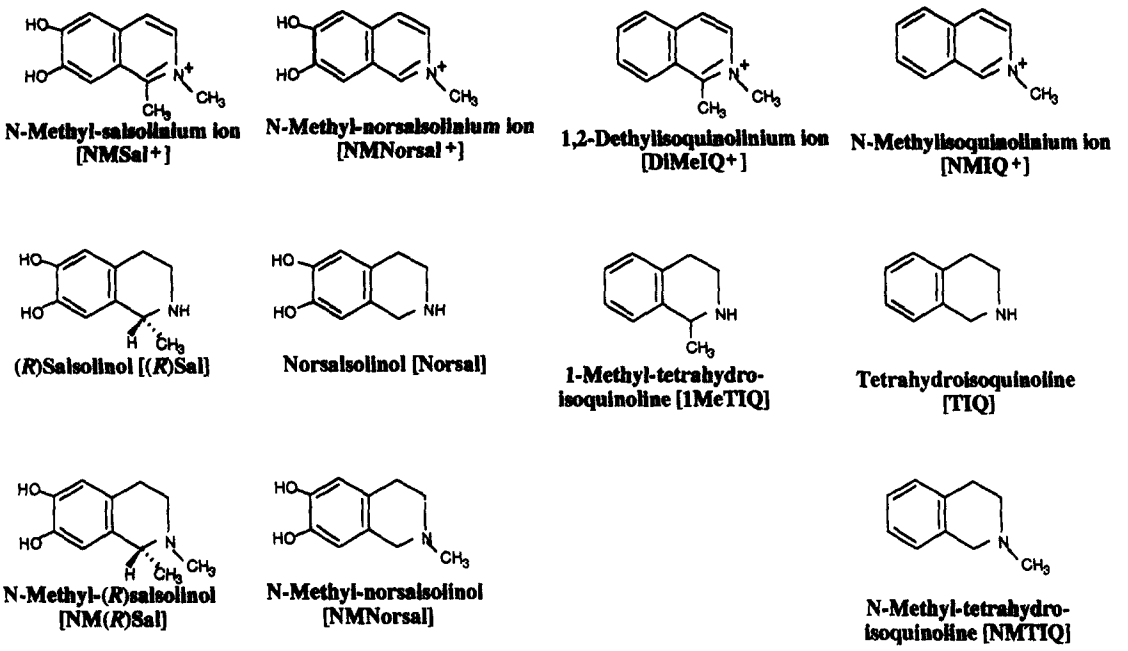

Fig 1. Chemical structures of *N*-methyl-6,7-dihydroxy-isoquinolinium ions, *N*-methylisoquinolinium ions and their precursors, 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinolines and 1,2,3,4-tetrahydroisoquinolines used for the experiments.

and 0.29 nmol/min/ mg protein, respectively. The effects of isoquinolines on MAO-A were examined and the activity–substrate concentration relationship is shown by Lineweaver–Burk's plot. Figure 2 shows that type A MAO was inhibited by (*R*)Sal and *N*-methylated derivatives. Inhibition was competitive to a substrate, and $NMSal^+$ was the most potent inhibitor among salsolinols. The K_i value of $NMSal^+$ was obtained as 9.21 ± 6.36 μM, which was much lower than the K_i values of (*R*)Sal and NM(*R*)Sal, as summarized in Table 1. The inhibition was proved to be reversible. By passing through a Sephadex G-25 column, the reduction of MAO activity by 100 μM $NMSal^+$ could be fully recovered. As summarized in Table 1, another catechol isoquinolinium ion, $NMNorsal^+$, was found to be a much less potent inhibitor to MAO-A than $NMSal^+$.

The effects of Sals on the activity of type B MAO

Table 1. Kinetics of type A monoamine oxidase and the effects of isoquinolines

Isoquinolines	K_m value (μM)	V_{max} value (nmol/min/mg protein)	K_i values* (μM)
Control	32 0±2.2	0 285±0 015	
$NMSal^+$	404 1±86 7	0 144±0.083	9 21±6 36
(*R*)Sal	78 5±18.0	0.173±0 035	75 9±21 0
NM(*R*)Sal	69 9±8 58	0 277±0.030	86 4±23 3
$NMNorsal^+$	87 5±29 6	0 141±0 043	44 4±7 9
$NMIQ^+$			40 5±4.9†
1,2-DiMeIQ$^+$	16.2±2 31	0 076±0 007	2 78±1 3‡
TIQ	85.3±9 44	0.264±0 026	55 4±14 4
NMTIQ	105 9±13 7	0.272±0 026	41 2±25 5
1-MetIQ	141.2±52.8	0 182±0.064	30 1±17 8

Each value represents mean ± SD.
* Inhibition was competitive to the substrate, except ****, which was non-competitive
† Naoi *et al*, 1987
‡ Inhibition was non-competitive, and the apparent V_{max} value was obtained with 10 μM DiMeIQ$^+$

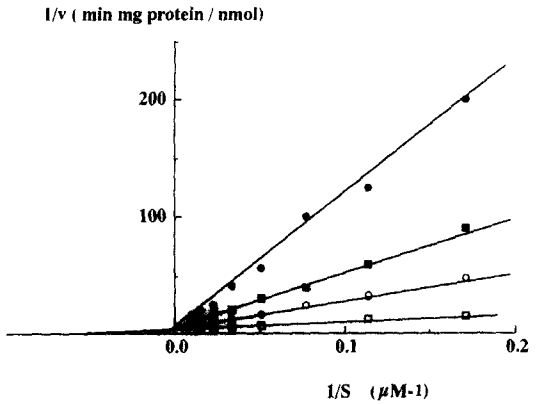

Fig 2 The effects of $NMSal^+$ and the related isoquinolines on MAO-A activity prepared from human brain synaptosomal mitochondria. The enzyme activity of MAO-A (0 87 mg protein) was measured in the absence and presence of isoquinolines. The reciprocal of the reaction velocity was plotted against that of the substrate concentration, according to Lineweaver and Burk Each value represents mean of the data obtained by duplicate measurements □, control, ○, activity measured with 100 μM (*R*)Sal, ■, with 100 μM NM(*R*)Sal, and ●, with 100 μM $NMSal^+$

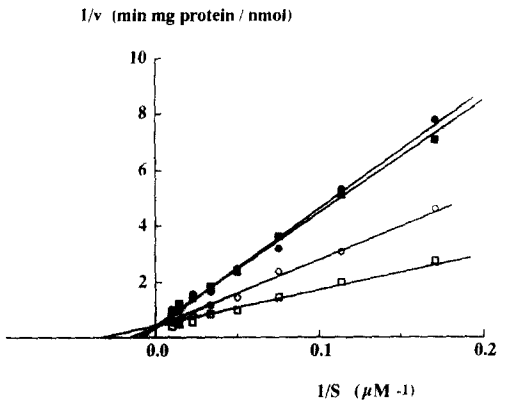

Fig. 3 The effects of $NMSal^+$ and related isoquinolines on MAO-B activity prepared from human brain synaptosomal mitochondria. The enzyme activity of MAO-B (0 87 mg protein) was measured in the absence and presence of isoquinolines The reciprocal of the reaction velocity was plotted against that of the substrate concentration, according to Lineweaver and Burk Each value represents the mean of the deta obtained by duplicate measurements □, control, ○, activity measured with 100 μM (*R*)Sal, ■, with 100 μM NM(*R*)Sal, and ●, with 100 μM $NMSal^+$

are presented in Fig. 3 and $NMSal^+$, (*R*)Sal and NM(*R*)Sal inhibited MAO-B activity in non-competition to the substrate. The K_i values of $NM(R)Sal^+$ and NM(*R*)Sal with MAO-B were much higher than those with MAO-A, as summarized in Table 1 The K_i value of (*R*)Sal to MAO-B was almost the same as that to MAO-A NMNorsal$^+$ did not inhibit MAO-B activity at concentrations up to 1 mM.

To clarify the chemical structure–activity relationship, isoquinolines without catechol structure were examined for their effects on MAO-A and -B activity The 1,2-Dimethylisoquinolinium ion ($1,2\text{-DiMeIQ}^+$) and *N*-methylisoquinolinium ion ($NMIQ^+$) are comparable to *N*-methylsalsolinium ion and *N*-methylnorsalsolinium ion (Fig. 1). $1,2\text{-DiMeIQ}^+$ was a very potent inhibitor of type A, with a K_i value of 2.78 μM The inhibition was non-competitive to the substrate, as shown in Fig. 4 Type B MAO was inhibited by these isoquinolinium ions in a non-competitive way to the substrate (Fig. 5) The K_i values to MAO-A of tetrahydroisoquinoline (TIQ) and 1- and 2-methylated tetrahydroisoquinoline (1-MeTIQ and NMTIQ) were almost the same. By comparison with isoquinolines with and without catechol structure, the presence of catechol structure increased the K_i values to MAO-A; (*R*)Sal vs 1-MeTIQ. Also concerning MAO-B TIQ and the derivatives were found to have much lower K_i values than catechol isoquinolines

Table 2 Kinetics of type B monoamine oxidase and the effects of isoquinolines

Isoquinolines	K_m value (μM)	V_{max} value (nmol/min/mg protein)	K_i values (μM)
Control	53 3±6 0		2 67±0 16
$NMSal^+$	85 1±19 3	2 11±0 43	77 8±14 4
(*R*)Sal	86 9±22 6	2 14±0 50	68 3±14 4
NM(*R*)Sal	67 4±7 12	3 00±0 19	433 3±262 7
$NMSal^+$	56 9±10 6	2 59±0 41	not inhibited
$NMIQ^+$			284 6±9 51†
$1,2\text{-DiMeIQ}^+$	45 9±1 44	1 81±0 05	8 87±2 56‡
TIQ	111 0±37 2	1 17±0 36	56 8±25 2
NMTIQ	116 1±47 4	1 85±0 15	24 8±8 27
1-MetIQ	77 8±16 8	2 16±0 41	116 4±13 0*

Inhibition was non-competitive or *competitive to the substrate The values are mean and SD of duplicate measurements of two independent experiments

† Naoi *et al*, 1987

‡ The enzyme activity was measured with 10 μM $DiMeIQ^+$

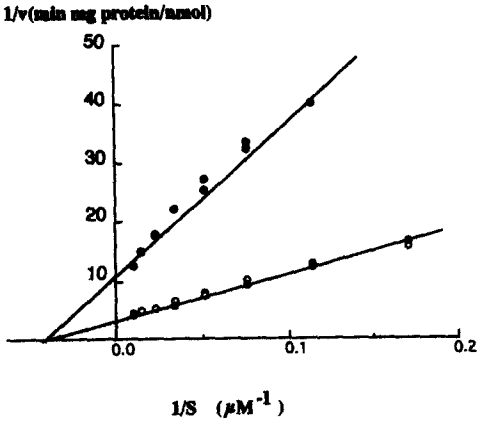

Fig. 4. The effects of 1,2-dimethylisoquinolinium on type A MAO prepared from human brain synaptosomes. The enzyme activity was measured in the absence and presence of 10 μM isoquinolilium ion. ○, control; ●, activity measured with 10 μM DiMeIQ+.

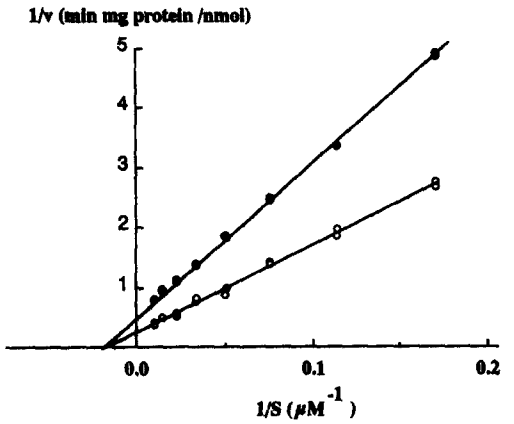

Fig. 5. The effects of 1,2-dimethylisoquinolinium ion on type B MAO prepared from human brain synaptosomes. The enzyme activity was measured in the absence and presence of 10 μM isoquinolinium ion. ○, control; ●, activity measured with 10 μM DiMeIQ+.

DISCUSSION

6,7-Dihydroxy-1,2,3,4-tetrahydroisoquinolines are synthesized from dopamine with pyruvic acid or an aldehyde in the brain (Strolin-Benedetti *et al.*, 1989; Dostert *et al.*, 1990), while most of tetrahydroisoquinolines without catechol structure are taken up from foods and transported into the brain (Niwa *et al.*, 1989). In the brain, the isoquinolines are *N*-methylated (Naoi *et al.*, 1989a; Maruyama *et al.*, 1992) and the enzymatic and non-enzymatic oxidation of *N*-methylated isoquinolines produces isoquinolinium ions. The structures of *N*-methyl tetrahydroisoquinolines and isoquinolinium ions are very similar to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 1-methyl-4-phenylpyridinium ion (MPP^+), as shown in Fig. 1. The enzymatic oxidation of MPTP into MPP^+ is considered to be the most important reaction to the dopaminergic neurotoxicity (Salach *et al.*, 1984). Also in the case of isoquinolines, the oxidized isoquinolinium ions were proved to increase the cytotoxicity to dopamine neurons (Naoi *et al.*, 1989c and 1994; Maruyama *et al.*, 1993b). More over, the isoquinolinium ions were proved to inhibit the enzyme activity of tyrosine hydroxylase [L-tyrosine, tetrahydrobiopterine:oxygen oxidoreductase (3-hydroxylating) EC 1. 14. 16. 2] (Hirata *et al.*, 1986; Naoi *et al.*, 1989c) and aromatic L-amino acid decarboxylase [aromatic L-amino acid carboxy-lyase, EC 1.4.1.28] (Naoi *et al.*, 1989c).

The inhibition of monoamine catabolism by isoquinolines may be neuroprotective under some conditions, as suggested by application of deprenyl. Salsolinol level is supposed to be increased by its metabolic precursors, dopamine, pyruvic acid and aldehydes in the brain, as in the case of L-DOPA treatment and alcoholism. The inhibition of type A MAO increases the extracellular levels of monoamine neurotransmitters such as norepinephrine and serotonin. Indeed, reduced catabolism of serotonin and dopamine was confirmed by perfusion of salsolinols in the rat striatum (Maruyama *et al.*, 1993a). By *in vivo* microdialysis in rat brains it was found that the major changes were increase in the extracellular levels of dopamine, norepinephrine and serotonin and reduction of those of monoamine metabolites (Maruyama *et al.*, 1993c). The *in vitro* data presented in this paper also suggest that the inhibition of MAO, especially of type A by isoquinolines, contributes to the reduction in monoamine catabolism. 1-Methyl-1,2,3,4-tetrahydroisoquinoline (1-MeTIQ) was found to reduce the behavioral changes elicited by MPTP and TIQ (Tasaki *et al.*, 1991). The neuroprotective activity of MAO inhibitors may be ascribed to economize the monoamine neurotransmitters and reduce the production of cytotoxic reactive oxygen species by the enzymatic oxidation. As naturally-occurring inhibitors of type A MAO, the *N*-methylated isoquinolinium ions may be one of the factors regulating monoamine levels in the brain under physiological and pathological conditions.

The effects of the chemical structure on the inhibitory activity were also examined, using the comparable isoquinolines with and without catechol structure. As shown above, the isoquinolinium ions

inhibited type A MAO more markedly than type B. The presence of methyl groups at the 1 and 2 positions of isoquinoline increased the inhibitory potency, as in the case of 1,2-dimethylisoquinolinium ion (1,2-DiMeIQ$^+$) and 1,2-dimethyl-6,7-dihydroxyisoquinolinium ion (NMSal$^+$). The presence of positive charge at the 2(N) position increases the inhibitory activity and the reduced form of isoquinolines have less potency to inhibit type A than the oxidized form. In the case of the reduced isoquinolines, the presence of a methyl group at 1 or 2 position does not seem to affect the affinity to MAO-A. The presence of catechol structure increased the selectivity of the inhibition, the catechol isoquinolinium ions inhibited type A MAO better than type B. NMSal$^+$ and NMNorsal$^+$ showed quite lower affinity to type B. NMSal$^+$ had a 10 times higher K_i value to MAO-B than MAO-A, and NMNorsal$^+$ virtually did not inhibit type B MAO. Also Norsal and NMNorsal were found to be poor inhibitors of MAO-B, as reported previously (Minami *et al*., 1993). On the other hand, 1,2-DiMeIQ$^+$ and the reduced isoquinolines without catechol, inhibit type B MAO almost with the same, or more, marked potency than type A. In the case of isoquinolines without catechol structure, TIQ and NMTIQ had almost same affinity to MAO-A and MAO-B, while the presence of one methyl group at C1 position of 1-MeTIQ and at C2 position of NMIQ$^+$ increased the affinity to type A. In conclusion, 1,2-dimethylisoquinolinium ion is a naturally-occurring potent inhibitor of type A monoamine oxidase. These results suggest that modification of the structure of isoquinolines by *in situ* metabolism may markedly change their biological activity in the brain.

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