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Quinolinic acid: an endogenous inhibitor specific for type B monoamine oxidase in human brain synaptosomes

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Quinolinic acid (QUIN), a well-known excitotoxin, was found to inhibit type B monoamine oxidase (MAO-B) in human brain synaptosomal mitochondria. By kinetic analysis, the inhibition of MAO-B activity by QUIN was competitive with the substrate, kynuramine. On the other hand, type A MAO (MAO-A) activity in human brain synaptosomal mitochondria and human placental mitochondria was not affected by QUIN. The selective inhibition of MAO-B by QUIN was confirmed using human liver mitochondria; only MAO-B was inhibited by QUIN and MAO-A was not inhibited. The inhibition was completely reversible. Among compounds structurally related to QUIN, 4-pyrimidine carboxaldehyde was the most potent substrate-competitive inhibitor of MAO-B, while 3-hydroxyanthranilic acid and xanthrenic acid, other metabolites of tryptophan, inhibited MAO non-competitively with the substrate. The inhibition of MAO-B by QUIN may be related to the causes of the neurotoxicity of QUIN.

Quinolinic acid (2,3-pyridine dicarboxylic acid, QUIN) is one of the metabolites of tryptophan [4], which occurs in human brain [11] and a structural analogue of neurotransmitter candidates such as L-glutamate and L-aspartate. QUIN is known as a potent excitotoxin; it causes neuronal excitation [10], seizures [6] and neuronal degeneration [8]. It is now proposed that QUIN may be involved in the etiology of some neurodegenerative disorders in humans [8]. By injection of nanomole amounts of QUIN into specific areas of the rat brain, a distinct pattern of nerve cell damage was observed, which was similar to that observed in Huntington's disease [3]. However, the mechanism of the damage by QUIN has not been well elucidated.

This paper describes for the first time that QUIN selectively inhibited type B monoamine oxidase (monoamine:oxygen oxidoreductase (deaminating), EC 1.4.3.4,

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MAO-B), and the possible functional significance of the MAO inhibition was discussed in terms of the toxicity of this compound in nerve cells.

Human brain synaptosomes were prepared as described in our previous paper [7]. MAO samples were prepared from human brain synaptosomes by solubilization with *n*-octyl glucopyranoside; synaptosomes were suspended in 10 mM potassium phosphate buffer, pH 7.4, to ca. 10 mg prot./ml, then washed with 0.1% *n*-octyl glucopyranoside, then MAO activity was solubilized with 1% *n*-octyl glucopyranoside. The solubilized sample was passed through a Sephadex G-25 column which was equilibrated and eluted with 10 mM potassium phosphate buffer, pH 7.4. QUIN and its structurally related compounds were purchased from Nakarai Chemicals (Kyoto). Clorgyline was kindly donated by May & Baker Co., and deprenyl by Dr. J. Knoll, Department of Pharmacology, Semmelweis University, Budapest, Hungary. The MAO activity was measured fluorometrically using kynuramine as substrate [5]. Type A MAO (MAO-A) or -B activity in human brain synaptosomal mitochondria was assayed in the presence of 0.1% *n*-octyl glucopyranoside and 1 μM deprenyl or clorgyline, respectively. In the case of the solubilized MAO sample, human liver and placental mitochondria, MAO activity was examined without preincubation, and in the case of human synaptosomes after 30 min preincubation at 37°C. The type of inhibition and the K_i values were obtained from kinetic data of the enzyme oxidation, using kynuramine at 8 different concentrations as the substrate. The data were plotted according to Lineweaver and Burk.

When the MAO activity in human brain synaptosomes and in the solubilized MAO

TABLE I

EFFECT OF QUIN ON MAO-A AND -B

Enzyme source	K_i with QUIN (μM)	K_m with kynuramine (μM)	V_{max} (nmol/min/mg prot.)
Human brain synaptosomal mitochondria[a]			
MAO-A[a]	No inhibition	31.7 ± 2.6[b]	0.39 ± 0.02
MAO-B	1410 ± 25	86.4 ± 8.1	1.38 ± 0.11
Solubilized human brain synaptosomal mitochondria			
MAO-A	No inhibition	26.3 ± 1.6	0.96 ± 0.04
MAO-B	565 ± 35	65.7 ± 6.0	4.60 ± 0.30
Human placental mitochondria			
MAO-A	No inhibition	68.5 ± 7.1	4.08 ± 0.31
Human liver mitochondria			
MAO-A	No inhibition	47.9 ± 2.5	2.79 ± 0.12
MAO-B	900 ± 28	65.2 ± 15.8	9.69 ± 0.43

[a]MAO-A or -B activity in human brain synaptosomes was measured in the presence of 1 μM deprenyl or clorgyline, respectively.

[b]Each value is the mean ± S.D. of triplicate measurements of 3 experiments.

sample was measured in the presence of QUIN, the enzyme activity was reduced in a dose-dependent way. Of the two types of MAO, only type B was inhibited by QUIN, and type A was not inhibited by QUIN at concentrations from 100 μM to 1 mM, as summarized in Table I. Using human placental mitochondria as another source of MAO-A, MAO-A was proved not to be inhibited by QUIN (Table I). By kinetic analysis, the inhibition of MAO-B with QUIN was found to be competitive with the substrate, kynuramine, as shown in Fig. 1. The selective inhibition of MAO-B by QUIN was confirmed using human liver mitochondria; only MAO-B was inhibited by QUIN, and MAO-A was not inhibited, as shown in Fig. 2. The inhibition was found to be immediate in the case of the solubilized MAO sample and of human liver and placental mitochondria, and only in the case of brain synaptosomes the inhibition was dependent on the preincubation time, and the maximal inhibition was obtained after 45 min preincubation at 37°C. As shown in Table I, the kinetic properties of brain synaptosomes did not change by solubilization procedure; the difference in the effect of the preincubation may be due to the difference of QUIN permeability into synaptosomes and to synaptosomal mitochondria. The inhibition by QUIN was confirmed to be completely reversible; after incubation of human brain synaptosomes with and without QUIN (300 μM) at 37°C for 45 min, the incubation mixture was dialyzed against 10 mM potassium phosphate buffer, pH 7.4, at 4°C overnight. MAO-B activity was obtained to be 0.75 ± 0.13 nmol/min/mg prot., which was virtually the same as the control value, 0.74 ± 0.01 nmol/min/mg protein.

In Table II, the effects of compounds structurally related to QUIN on MAO activity are summarized. Among the compounds tested, 4-pyridine carboxaldehyde was the most potent substrate-competitive inhibitor of MAO-B, and then QUIN. Out of

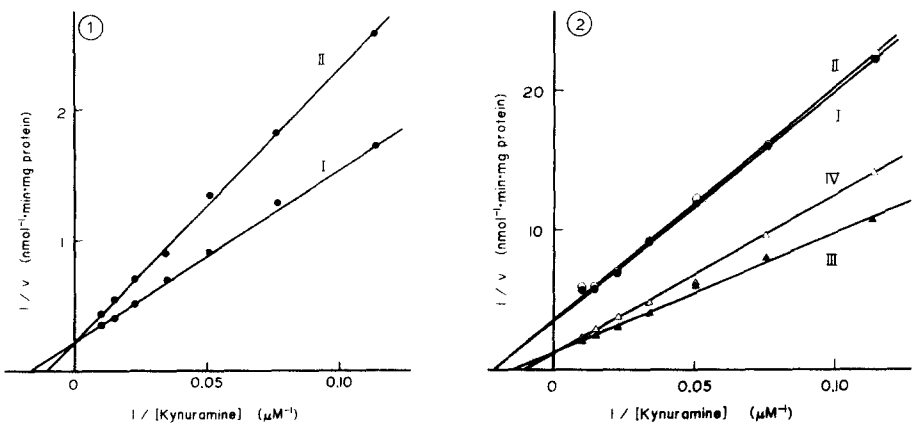

Fig. 1. Double-reciprocal plots of MAO-B activity in the solubilized MAO sample from human brain synaptosomes in the absence and presence of QUIN. MAO-B activity in the solubilized MAO sample (2 μg prot.) was measured in the presence of 1 μM clorgyline. I, Control; II, in the presence of 300 μM Quin.

Fig. 2. Double-reciprocal plots of MAO-A and -B activity in human liver mitochondria in the absence and presence of QUIN. MAO-A and -B activity in human liver mitochondria (300 μg prot.) was measured in the presence of 1 μM deprenyl or clorgyline, respectively. I, MAO-A, control; II, MAO-A in the presence of 300 μM QUIN; III, MAO-B, control; IV, MAO-B in the presence of 300 μM QUIN.

TABLE II

EFFECTS OF QUIN AND ITS ANALOGUES ON MAO-B ACTIVITY IN HUMAN BRAIN SYNAPTOSOMAL MITOCHONDRIA

Compound	K_i value of MAO-B (μM)
Quinolinic acid	1410 ± 25[a]
2,5-Pyridine dicarboxylic acid	1820 ± 170
2,6-Pyridine dicarboxylic acid	7500 ± 120
2-Pyridinecarboxaldehyde	1870 ± 135
4-Pyridinecarboxaldehyde	653 ± 11
Picolinic acid	No inhibition
Nicotinic acid	3970 ± 110
3-Hydroxyanthranilic acid	935 ± 40[b]
Xanthurenic acid	160 ± 24[b]
Kainic acid	No inhibition
Ibotenic acid	No inhibition

[a]Each value is the mean ± S.D. of duplicate measurements of two experiments. MAO-B activity was measured in the presence of 1 μM clorgyline.
[b]The inhibition was non-competitive with the substrate.

3 isomers of pyridine dicarboxylic acid, pyridine 2,3-dicarboxylic acid, QUIN, was the most effective inhibitor of MAO-B. The inhibition of all these compounds was competitive with the substrate, and MAO-A in human brain synaptosomes and placental mitochondria was not inhibited by these compounds. As shown in Fig. 3, kainic acid (KA) and ibotenic acid (IA), other well-known excitotoxins, and picolinic acid, 2-pyridine carboxylic acid, were proved to inhibit neither MAO-B nor -A. Other metabolic precursors or metabolically related compounds were also tested. 3-Hydroxyanthranilic acid and xanthurenic acid were found to inhibit MAO-B non-competitively with the substrate, as shown in Fig. 3.

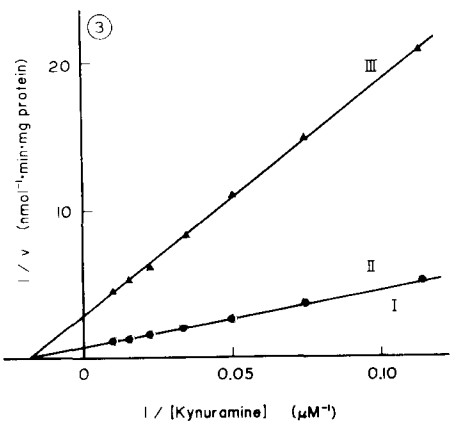

Fig. 3. Double-reciprocal plots of MAO-B in human brain synaptosomes in the absence and presence of kainic acid and xanthurenic acid. MAO-B activity in human brain synaptosomes (450 μg prot.) was measured in the presence of 1 μM clorgyline, after 30 min incubation with the inhibitor. I, control; II, in the presence of 300 μM kainic acid; III, in the presence of 300 μM xanthurenic acid.

These data indicate that QUIN is an inhibitor specific for type B MAO: QUIN inhibited MAO-B competitively with the substrate and did not affect MAO-A. Even though numerous compounds have been reported, QUIN is the first naturally occurring inhibitor of MAO-B. MAO activity in the brain regulates the levels of biogenic amines, but abnormality in MAO activity in neurodegenerative diseases has not been well studied. The data on the inhibition of MAO-B by QUIN suggest that a perturbation of catecholamines and indoleamines in the brain may occur in some diseases. QUIN is known to induce a highly significant alteration in the physical state of membrane protein in erythrocyte ghosts, but that of membrane lipids is unaffected [1]. The inhibition by QUIN may be due to a conformational change in the active site of the enzyme, as suggested by its substrate-competitive binding with the enzyme. Regarding the death of the nerve cells by QUIN, we should further demonstrate the causal relation between the MAO inhibition and the cell damage. However, QUIN may be an endogenous inhibitor or modulator of MAO-B in human brain to regulate the levels of biogenic amines.

Neuronal lesions caused by intracerebral injection of QUIN, KA and IA are all of the axon-sparing type [9]. But, they seem to differ in their mechanism; the type of QUIN receptor on the presynaptic membrane is different from the receptors of KA and IA, and kynurenic acid selectively protects against the neurotoxicity and seizures in rat by QUIN [2]. Our data on the selective inhibition of MAO-B by QUIN further suggest that mechanistic differences may occur among such excitotoxins.

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