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Polish Journal of Pharmacology
Pol. J. Pharmacol., 2004, 56, 727–734
ISSN 1230-6002

INHIBITION OF RODENT BRAIN MONOAMINE OXIDASE AND TYROSINE HYDROXYLASE BY ENDOGENOUS COMPOUNDS – 1,2,3,4-TETRAHYDROISOQUINOLINE ALKALOIDS

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Inhibition of rodent brain monoamine oxidase and tyrosine hydroxylase by endogenous compounds – 1,2,3,4-tetrahydroisoquinoline alkaloids. A. PATSENKA, L. ANTKIEWICZ-MICHALUK. Pol. J. Pharmacol., 2004, 56, 727–734.

Four different noncatecholic and one catecholic tetrahydroisoquinolines (TIQs), cyclic condensation derivatives of β-phenylethylamine and dopamine with aldehydes or keto acids, were examined for the inhibition of rat and mouse brain monoamine oxidase (MAO) and rat striatum tyrosine hydroxylase (TH) activity. Simple noncatecholic TIQs were found to act as moderate (TIQ, N-methyl-TIQ, 1-methyl-TIQ) or weak (1-benzyl-TIQ), MAO B and MAO A inhibitors. 1-Methyl-TIQ inhibited more potently MAO-A than MAO-B; the similar but more modest effect was exerted by salsolinol. Only salsolinol markedly inhibited TH activity, being competitive with the enzyme biopterin cofactor. The inhibition of MAO and TH by TIQs is discussed in relation to their ability to regulate monoamine metabolism.

Key words: *monoamine oxidase, tyrosine hydroxylase, inhibition of enzyme activity, tetrahydroisoquinoline derivatives*

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Abbreviations: BBB – *blood-brain barrier*, BH_4 – *5,6,7,8-tetrahydrobiopterin*, CSF – *cerebrospinal fluid*, 5-HT – *5-hydroxytryptamine*, MAO – *monoamine oxidase*, MPTP – *1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine*, β-PEA – *2-phenylethylamine*, TH – *tyrosine hydroxylase*, THP – *1,2,3,4-tetrahydropapaveroline*, TIQ *1,2,3,4- tetrahydroisoquinoline*

INTRODUCTION

In physiological conditions catecholamines condense with a variety of simple and biogenic aldehydes to form tetrahydroisoquinoline (TIQ) alkaloids [7, 9]. The presence of TIQs in mammals including humans has been widely reported [26, 28]. Using high-performance liquid chromatography (HPLC) with electrochemical, fluorescence or gas chromatography with mass spectrometry detection many authors have shown the presence of TIQ derivatives such as TIQ, 1-methyl-TIQ (1MeTIQ), N-methyl-TIQ (NMeTIQ), 1-benzyl-TIQ (1BnTIQ), 1-methyl-6,7,-dihydroxy-TIQ (salsolinol) and tetrahydropapaveroline (THP) in rodent and human brain (see a review by Nagatsu [24]). On the other hand, TIQs are also widely distributed in the environment, being present in many plants and foodstuffs, such as port wine, bananas, beer and milk [17, 27]. The exogenously administered TIQs easily cross the blood-brain barrier (BBB) and migrate into the brain, producing behavioral and biochemical effects in monoamine systems [2, 3, 11, 21]. Some of them, e.g. salsolinol, a product of the condensation of dopamine (DA) with acetaldehyde and/or pyruvate, have aroused considerable interest as it may be involved in the pathogenesis of alcoholism and neurodegeneration [24]. Other-than-aromatic amines (phenylethylamines) may also undergo condensation with aldehydes yielding such TIQs as TIQ and 1MeTIQ. These alkaloids are presumably activated *via* N-methylation and are oxidized by monoamine oxidase (MAO) to form a neurotoxic N-methylisoquinolinium ion, the same as MPP^+ (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridinium ion) from 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxin, which produces a clinical syndrome similar to Parkinson's disease. Indeed, it has been shown that salsolinol is a weak substrate for N-methyltransferase in the rat substantia nigra, but its endogenous concentration is minute [26]. Some TIQs, such as 1BnTIQ, have been found to be toxic to dopaminergic neurons and their concentration has been shown to increase in parkinsonian brains or cerebrospinal fluid (CSF) [12]. Others, such as 1MeTIQ, show a neuroprotective effect against the rotenone-induced neurodegeneration and mortality in rats [1, 5].

Tyrosine hydroxylase (TH) and MAO are crucial enzymes for catecholaminergic neurons. The first step of catecholamines synthesis is catalyzed by TH, a rate-limiting regulatory enzyme. TH is an iron-containing 3-monooxygenase (tyrosine-tetrahydrobiopteridine (BH_4): oxygen oxidoreductase, EC 1.14.16.2, TH) expressed in catecholamine-neurons in mammalian brain, adrenal medulla and peripheral sympathetic nerve terminals. The main catabolic enzyme for brain monoamines is monoamine oxidase (amine: oxygen oxidoreductase, flavin-containing, EC 1.4.3.4, MAO). It occurs usually as the dimer and is classified into isoenzymes A and B with a different substrates and inhibitors, which are encoded by two different genes. These enzymes can also produce hydrogen peroxide and other reactive oxygen species as additional products of their reactions; oxidative deamination also results in the production of very reactive biogenic aldehydes [8, 13, 35].

Salsolinol derivatives have been reported to inhibit the activity of tryptophan hydroxylase and TH [30, 34, 40], some hydroxylated TIQs have shown to affect MAO activity [6, 22, 23].

However, till now it has not been clarified whether TIQs without a catechol structure can disturb the monoamine synthesizing and metabolizing enzymes. The aim of the present study was to determine the effect of four noncatecholic TIQs (TIQ, 1MeTIQ, NMeTIQ and 1BnTIQ) on the activity of MAO and TH and compare them with salsolinol.

MATERIALS and METHODS

Chemicals and animals

L-[3,5-H] tyrosine (51 Ci/mmol), 5-hydroxy-3-indolyl [^{14}C] ethyl-2-amine creatinine sulfate ([^{14}C]-5-HT, 56.7 mCi/mmol) and 2-phenyl[^{14}C]ethylamine hydrochloride ([^{14}C-β-PEA, 57 mCi/mmol) was purchased from Amersham (UK). L-tyrosine, 5-HT, β-PEA, α-methyl-*p*-tyrosine, salsolinol. HCl, TIQ, THP, BH_4, dithiotreitol, bovine serum albumin (BSA) and catalase were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Clor-

giline and L-deprenyl came from RBI (MA, USA). 1MeTIQ, NMeTIQ and 1BnTIQ were synthesized in the Department of Medicinal Chemistry of the Institute of Pharmacology, Polish Academy of Sciences in Kraków. All other reagents were of analytical grade.

Male mice (18–20 g) and Wistar rats (240–280 g) were used for tissue preparation. All the procedures were carried out according to the National Institutes of Health Guide for Care and Use of Laboratory Animals, and were approved by the Institute's Internal Bioethics Commission.

Determination of monoamine oxidase activity

MAO-A and MAO-B activities were determined by a radiochemical assay according to the method of Tipton and Yodim [38], with further modifications.

5-Hydroxy-3-indolyl [^{14}C] ethyl-2-amine creatinine sulfate and 2-phenyl[^{14}C]ethylamine hydrochloride were used as the substrates for MAO-A and MAO-B, respectively. Animals (rats or mice) were sacrificed by decapitation, brain tissue was removed, dissected and frozen till further assayed. Pieces of the frontal cortex, striatum and brainstem of rats or whole brains, except for the cerebellum of mice, were homogenized in 50 mM phosphate buffer (pH 7.4, 10% w/v) using a Teflon homogenizer. All manipulations were carried out at 4°C. Homogenates were centrifuged at 1000 × g for 5 min; the sediment was discarded and the supernatant was recentrifuged at 11800 × g for 30 min, at +4°C. The pellet (mitochondrial fraction) was resuspended in the same buffer to final protein concentration of 1.0–1.5 mg/ml, determined by Lowry's et al. method [15].

The preincubation medium (50 mM phosphate buffer, pH 7.4) contained 100 µl of displacing solution with TIQ-alkaloids, ranging from 1.0 µM to 1.0 mM, or the MAO inhibitors clorgiline or L-deprenyl (0.1 nM–0.5 µM) and 100 µl of resuspended pellet. After 10 min of preincubation at 37°C with shaking, the reaction was started by adding 50 µl of a radioactive substrate, 100 µM of [^{14}C]-5-HT or 10 µM of [^{14}C]- β-PEA (final concentrations), and was carried out at 37°C for 30 and 10 min, respectively. The reaction was terminated by adding 50 µl of 4 M HCl and cooling. Reaction products were extracted by vortex with 2 ml of ethyl acetate/toluene (1:1, v/v) (POCH, Poland) and 1 ml of the organic layer was withdrawn and mixed with 4 ml of the scintillation fluid. Radioactivity was determined by liquid scintillation spectrometry (Beckman LS 6500 counter). Data were expressed in nmol/min/mg of protein of 5-TH- and β-PEA-oxidized products for MAO-A and B, respectively.

Determination of tyrosine hydroxylase activity

TH activity was determined by a radiochemical assay according to the method of Rejnhard et al. [33] with further modifications.

The tissue was prepared in the same way as for the MAO assay. After the last centrifugation, the supernatant was used in TH assay and the protein concentration was about 3.0 mg/ml. TH reactions were carried out at a total volume of 100 µl in Brand 1.5 ml polyethylene tubes. Reaction mixture contained the following components: 75–80 µg of protein; MES buffer, 50 mM, pH 6.0; dithiothreitol, 5 mM; catalase (Sigma C-100, 47 000 U/mg), 90 µg; L-tyrosine/L-[ring-3,5-^{3}H]tyrosine, 50 µM/1 µCi per sample; (6R)-L-erythro-5,6,7,8,-tetrahydrobiopterin(BH_4), 125–500 µM. Blank values, obtained by omitting BH, equaled the blank values when tissue was discarded. Thus, the last blank was routinely used. The reaction was initiated by adding BH_4, incubated at 37°C for 20 min, and was terminated with 0.7 ml of a stirred suspension of 7.5% (w/v) charcoal (POCh, Poland) in 0.1 M HCl. The mixtures were then rapidly stirred for 20 s and centrifuged at 10 000g for 3 min. Aliquots 0.4 ml of the clear supernatant were transferred to 5 ml scintillation vials, and 4.0 ml of Aquascint (BIOCARE, Poland) were added. TH utilizes O_2 to produce DOPA and ^{3}H-H_2O from ^{3}H-tyrosine. Unreacted tyrosine and catecholamines were absorbed with an aqueous slurry of activated charcoal, and the released ^{3}H-H_2O was analyzed by liquid scintillation counting. Data were normalized for total protein and were expressed as nanomoles of DOPA formed per minute per milligram of protein. The protein was assayed using bovine serum albumin as a standard by the method of Lowry et al. [15].

The data are expressed as means ± SE from three independent experiments. The results (IC_{50}) were assayed using the Prism Pad 4 program (nonlinear regression).

RESULTS

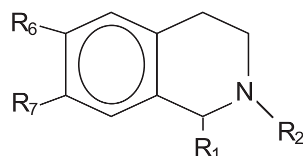
Enzyme activity

Figure 1 shows the chemical structure of TIQs used in this study. In order to optimize the analytical assay for the determination of MAO-A, B and TH activity, we studied previously different reaction times and different substrate concentration. The optimal conditions were used, and V_{max} values were summarized in Table 1. The regional distribution of MAO-A activity was the same in the striatum, frontal cortex and the brainstem of rat brain. MAO-B activity was increased in the brainstem. MAO-A activity was more than twice as high as that of MAO-B in rat and mouse brain.

In addition, the effects of specific isoenzyme inhibitors were analyzed. Clorgiline inhibited MAO-A at IC_{50} = 2.2 nM and 1.95 nM, and L-deprenyl inhibited MAO-B at IC_{50} =14 nM and 15 nM for mouse and rat brain, respectively. α-Methyl-*p*-tyrosine potently inhibited striatal TH activity (67.8% of the inhibition in a 0.01 mM concentration).

Effects of tetrahydroisoquinoline alkaloids on MAO activity

TIQs inhibited enzyme activity in a dose-dependent manner as shown in Figure 2. IC_{50} values (the compound concentration that produced 50% inhibition of the enzyme activity) for each alkaloid were almost identical in mouse and rat brain and in the structures of rat brain studied (Tab. 2). Salsolinol inhibited MAO-A activity the most effectively in rat striatum, and less efficiently in other rat and mouse brain structures. TIQ and NMeTIQ were the most potent MAO-B inhibitors in comparison with other alkaloids, but IC_{50} values were the same in both MAO-A and B only for TIQ. The range of IC_{50} values at 30 μM may be recordered as a moderate inhibition potency [6, 37]. 1MeTIQ had the same IC_{50} values with MAO-A (29–32, 33 μM) but five-fold higher IC_{50} values with MAO-B (164-230, 160 μM) in rat and mouse brains. Salsolinol produced similar but modest effects compared to 1MeTIQ (IC_{50} = 62–230, 170 μM and 582–1000, 1000 μM, with MAO-A and B of rat and mouse brain, respectively). It was assumed that salsolinol lacked the inhibition potency in relation to MAO-B. High IC_{50} of 1BnTIQ pointed to its weak inhibition potency in relation to both MAO isoenzymes.



	R1	R2	R6	R7
TIQ	H	H	H	H
1 Me TIQ	CH_3	H	H	H
N Me TIQ	H	CH_3	H	H
1 Bn TIQ	CH_2-C_6H_6	H	H	H
Salsolinol	CH	H_3	OH	OH

Fig. 1. Chemical structures of tetrahydroisoquinoline (TIQ) alkaloids

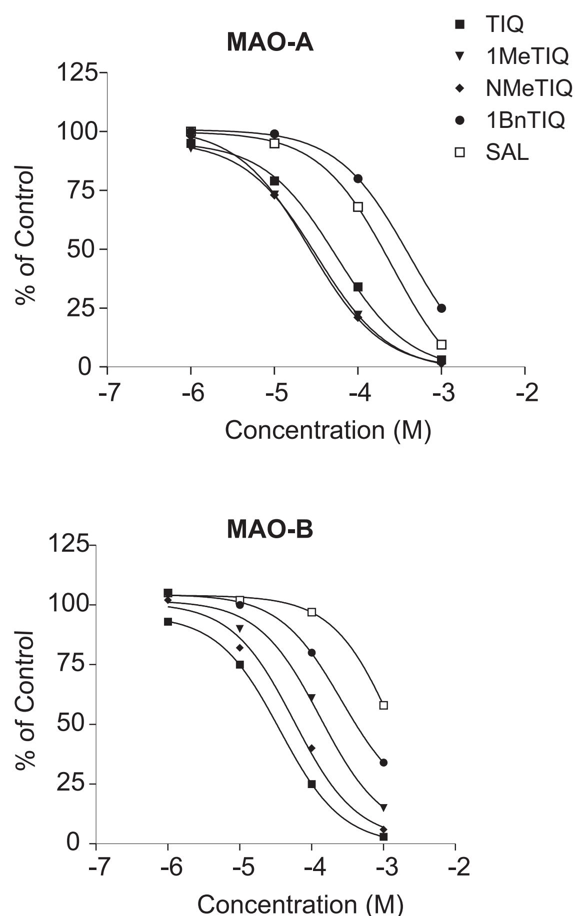


Fig. 2. Inhibition of mouse brain monoamine oxidase (MAO) A and B by tetrahydroisoquinoline (TIQ) alkaloids. IC_{50} (μM): TIQ – 50 (MAO-A) and 35 (MAO-B); N-methyl-TIQ – (NMeTIQ) 29 and 62; 1-methyl-TIQ (1MeTIQ) = 33 and 160; 1-benzyl-TIQ – (1BnTIQ) 375 and 470; salsolinol – (SAL)170 and > 1000, respectively. Values are the means of three independent experiments. TIQs concentrations were used from 1 μM to 1 mM

Table 1. Monoamine oxidase (MAO) and tyrosine hydroxylase (TH) activity in rodent (V_{max}, nmol/min/mg of protein)

		MAO-A	MAO-B	TH
Rat brain	striatum	2.17 ± 0.11	0.48 ± 0.03	0.23 ± 0.02
	frontal cortex	2.34 ± 0.13	0.68 ± 0.07	–
	brain stem	2.30 ± 0.09	0.98 ± 0.06	–
Mouse brain (without cerebellum)		1.46 ± 0.06	0.58 ± 0.03	

Table 2. Monoamine oxidase (MAO) A and B inhibitory activity (IC_{50} values, M) of tetrahydroisoquinolines (TIQ) in rat brain regions

		MAO-A	MAO-B
Striatum	TIQ	23	34
	1 Me TIQ	31	164
	N Me TIQ	38	67
	1 Bn TIQ	350	548
	Salsolinol	62	585
Frontal cortex	TIQ	47	40
	1 Me TIQ	32	230
	N Me TIQ	39	95
	1 Bn TIQ	235	420
	Salsolinol	230	1000
Brainstem	TIQ	27	43
	1 Me TIQ	29	171
	N Me TIQ	37	76
	1 Bn TIQ	320	640
	Salsolinol	195	1000

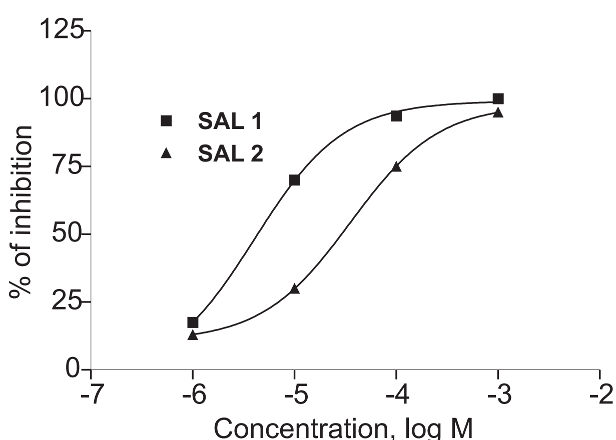


Fig. 3. Inhibition of rat striatum tyrosine hydroxylase by salsolinol (SAL) with different concentrations of tetrahydrobiopterin (BH_4). SAL 1: 250 μM BH_4, IC_{50} (μM) = 4.1; SAL 2: 500 μM BH_4, IC_{50} (μM) = 35.4. Values are the means of three independent experiments. TIQs concentrations were used from 1 μM to 1 mM

Effects of tetrahydroisoquinoline alkaloids on TH activity

Only salsolinol was effective as an inhibitor of TH activity. IC_{50} values for all four nonhydroxylated TIQs exceeded 1000 μM (data not shown). Figure 3 shows the curves of TH activity inhibition by salsolinol at two different concentrations of tetrahydrobiopterin (500 μM and 250 μM) and IC_{50} for salsolinol (35.4 and 4.1 μM, respectively). Additionally, we tested an other hydroxylated complex alkaloid THP, a product of DA and dopaldehyde condensation. The obtained results show the same inhibition potency of THP (IC_{50} =7.5 μM) in relation to rat striatum TH.

DISCUSSION

In this report, we consider the possibility that TIQs derived from μ-PEA can affect catecholamine biosynthesis and catabolism by different way with well-known DA-derived alkaloids. The inhibition of MAO by hydroxylated TIQs and their N-methylated products has been examined by several authors in *in vitro* and *in vivo* experiments [6, 18, 22, 23]. Thull et al. [37] tested a few noncatecholic TIQs (TIQ, NMeTIQ), and demonstrated a reversible and competitive type of the inhibition of MAO activity in rat brain. They confirmed earlier study [6] and indicated that TIQ-alkaloids were not substrates of MAO-A or B, even when they were used in relatively high concentrations. In our report, we have compared effects of salsolinol with those of noncatecholic, simple and substituted TIQs. Simple noncatecholic TIQs were similarly active towards mouse and rat MAO isoenzymes. In contrast to Thull et al. [37], our results revealed more potent inhibition of MAO-A and B by TIQ and NMeTIQ (IC_{50} values were not higher than 29–39 and 34–95 μM, with MAO-A and MAO-B, respectively). The presence of hydroxyl groups at position 6 and 7 of aromatic ring is not required for an inhibitory

effect, as was assumed by Naoi et al. [26] on the basis of Thull's et al. results. The above discrepancy may be explained by the different methods used. We used a high specific radiochemical method with different labeled substrates for each form of the isoenzyme. Kynuramine fluorimetric assay used by Thull et al. needs to inhibit part of enzyme activity by specific irreversible inhibitor to analyze another part of isoenzyme (clorgiline for MAO-B and selegiline for MAO-A). It is very difficult to calculate the appropriate concentrations of these compounds. Thus, administration of a low dose of L-deprenyl (2.5 mg/kg) inhibited MAO-B activity by 90%, but also MAO-A activity by 40–60% [41]. In addition, the inhibition of MAO-B is attenuated when the hydrogen at position 1 is substituted with methyl group (in 1MeTIQ and salsolinol) or with a more complex group (1BnTIQ). This is in agreement with a recent study into MAO molecular structure [16], whose authors determined the crystal structure of rat enzyme, compared isoforms A and B, and explained the role of MAO-B Tyr326 in blocking the access of the hydroxyl group of substrate to the binding pocket.

It is well known that MAO-A plays a leading role in rodent monoamine catabolism. MAO-A knock-out (KO) mice exhibited aggressive behavior and increased levels of 5-HT, norepinephrine and DA, whereas MAO-B KO mice showed an increased β-PEA level only [10, 31]. However, a high dose of L-DOPA (100 mg/kg, *ip*) injected to MAO-B KO mice produced a higher level of DA than that in wild-type mice, that difference being abolished by pretreating the wild-type animals with L-deprenyl [10]. It is assumed that MAO-B is involved in monoamine metabolism if monoamine levels are abnormally elevated. The elevated turnover of catecholamines, in particular DA, is regarded as oxidative stress factor which contributes to the progression of neurodegenerative disorders. The consequence of MAO activity is the exposure of neuronal cells to an increased flux of hydrogen peroxide and aldehyde/quinone products of MAO reaction [8]. In turn, elevated reactive oxygen species may inactivate the enzyme itself [35]. On the other hand, the inhibition of MAO-B produces a "cheese" effect and potentiates the evoked stereotypic behavior [41]. From the above viewpoint 1MeTIQ seems to be of interest as a compound with moderate affinity for MAO-A and lower affinity for MAO-B. Indeed, it possesses distinct neuroprotective properties [1, 2, 11].

It has been proposed also that cyano-products of TIQ interaction with cigarette smoke may act as MAO inhibitors and may contribute in neuroprotective effect of tobacco smoking against Parkinson's disease [20, 36]. Another interesting application is the use of preferential reversible MAO-A inhibitors to attenuate the reward and dependence of drug abuse [19]. 1MeTIQ may be one of them.

Our results corroborate the inhibition of TH activity and competitive interaction of salsolinol with binding site for pteridine cofactor, which was demonstrated earlier for TH and tryptophan hydroxylase [30, 34]. Noncatecholic TIQs are inactive in relation to TH. Possibly, the availability of hydroxyl and/or carboxyl groups in the chemical structure are necessary to develop inhibitory activity. This have been confirmed by our experiment with THP and by other studies with norsalsolinol derivatives [34]. In view of the fact that the physiological concentration of brain BH_4 is about 100 μM [13], *in vivo* salsolinol may potentiate its effect on TH. However, there remains a problem of its endogenous brain concentration and BBB permeability. In contrast to noncatecholic TIQs which easily penetrate BBB, hydrophilic chemical structure and fast metabolism of salsolinol remain the question opened [29]. The fact that salsolinol interacts with BH_4 needs to be further investigate, because of the pivotal role of the pteridine cofactor in the functioning of not only monoamine synthesizing enzymes, but also nitric oxide synthase, and due to its being a crucial factor in the resistance of dopaminergic neurons [25].

In conclusion, the role of TIQs as moderate to low reversible inhibitors of both MAO isoenzymes may present the group of endogenous compounds with very profitable mechanism of action, as neuroprotective activity what was presented for 1MeTIQ [1, 5]. This hypothesis is in agreement with other reports which have shown the lack of neurotoxicity of TIQ in rodents [14, 32]. However, it should be mentioned, that some of TIQ derivatives as 1BnTIQ or salsolinol, the compounds with low affinity to MAO and inhibitory activity to TH (salsolinol) may produce moderate neurotoxic effect presented both in *in vitro* and *in vivo* studies [4, 12, 26].

Finally, the results obtained in the present study confirm our concept that endogenous TIQ alkaloids may act as physiological regulators and feed-back

controllers of brain monoamines, in particular DA neurotransmitter system [2, 3, 4, 39].

Acknowlegments. Thanks are due to Ms Elżbieta Smolak for the English correction of the paper. This study was supported by the statutory funds of the Institute of Pharmacology, Polish Academy of Sciences Kraków, Poland.

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Received: *November 2, 2004; in revised form: November, 23, 2004.*