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Quinolinate-like neurotoxicity produced by aminooxyacetic acid in rat striatum*,**

W. A. Turski, E. Urbańska, M. Sieklucka, and **C. Ikonomidou**

Department of Pharmacology, Medical School, Lublin, Poland

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Summary. The endogenous tryptophan metabolite quinolinic acid elicits in rodent brain a pattern of neuronal degeneration which resembles that caused by L-glutamate. Its qualities as a neurotoxic agent raised the hypothesis that quinolinic acid might be involved in the pathogenesis of human neurodegenerative disorders. Kynurenic acid, another endogenous tryptophan metabolite and preferential N-methyl-D-aspartate (NMDA) antagonist, has been shown to block quinolinic acid neurotoxicity. Here we report that microinjections of aminooxyacetic acid (AOAA), an inhibitor of kynurenine transaminase and of other pyridoxal phosphate-dependent enzymes, into the rat striatum produce neuronal damage resembling that caused by quinolinic acid. AOAA-induced striatal lesions can be prevented by kynurenic acid and the selective NMDA antagonist 2-amino-7-phosphonoheptanoic acid. These results suggest that AOAA produces excitotoxic lesions by depleting brain concentrations of kynurenic acid (inhibition of synthetic enzyme) or due to impairment of intracellular energy metabolism (depletion of cell energy resources). The concept of deficient neuroprotection due to metabolic defects might help to clarify the pathogenesis of human neurodegenerative disorders and to develop strategies that may be useful in their treatment.

Keywords: Amino acids – Aminooxyacetic acid – Neostriatum – Kynurenic acid – N-Methyl-D-aspartic acid – Rat

It has been recognized for some years that excitatory amino acids (EAA) such as L-glutamate and L-aspartate can destroy neurons in the mammalian CNS by excessive activation of specific receptors [1, 2]. Subsequently, the hypothesis

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was raised that hyperfunction of the body's own excitotoxins might cause neuronal degeneration in several human neurological disorders [3, 4, 5, 6]. Alternatively, it was suggested that relief of the Mg^{2+} block of N-methyl-D-aspartate (NMDA) receptor channels due to energy deprivation may enable excitatory amino acids to act persistently at their receptors, resulting in the opening of ion channels, and enabling L-glutamate to become neurotoxic [7]. Quinolinic acid (QUIN), an endogenous tryptophan metabolite [8], has attracted interest because of its ability to produce axon-sparing lesions of the rodent striatum with behavioral, neurochemical and neuropathological characteristics similar to those observed in Huntington's disease [9, 10, 11, 12]. The NMDA receptors seem to be responsible for mediating toxicity of QUIN since competitive NMDA antagonists such as 2-amino-7-phosphonoheptanoate (AP7) block cell death induced by QUIN in the striatum [9]. Kynurenic acid (KYNA), another endogenous tryptophan metabolite [13], has been shown to possess antagonistic properties at EAA receptors and block QUIN-neurotoxicity [14, 15, 16, 17]. Subsequently, an overabundance of QUIN or a hypofunction of KYNA has been hypothetically linked to the pathogenesis of human neurodegenerative disorders [15, 18, 19]. However, no evidence has been presented so far to support the proposal that hypofunction of KYNA in the brain may result in excitotoxic cell death. Recent work suggests that KYNA may indeed possess physiological significance in the brain. KYNA has been identified in human brain tissue where it occurs at concentrations of 1 μM [20, 21]. Additional evidence for the synthesis of KYNA in rat brain slices upon exposure to L-kynurenine has been reported [22]. Interestingly, aminooxyacetic acid (AOAA) is an inhibitor of the KYNA synthesizing enzyme kynurenine transaminase [19].

On the other hand, it has been suggested that L-glutamate acting via NMDA receptors mediates neurotoxicity when intracellular energy metabolism is reduced [7]. It has been shown that excitotoxic lesions, such as those induced by potassium cyanide in the chick retina, are blockable by glutamate antagonists and can be induced in the absence of detectable increase of L-glutamate release [23]. Similar mechanism may explain toxicity of MPP^+ (1-methyl-4-phenylpyridinium ion) in the rat substantia nigra [24]. MPP^+ toxicity is blockable by glutamate antagonists [24] and may be due to complex I inhibition and consequent depletion of energy resources in the neurons [25]. Importantly, a depletion of intracellular energy resources has been long suggested as a possible mechanism of convulsant action of AOAA in rodents [26].

Therefore the present study was designed to investigate the consequences of the action of AOAA, which is a non-selective inhibitor of several transaminases [19] but may also interfere with energy metabolism depleting cell energy supplies [26], in the rat brain.

Materials and methods

Male and female Wistar rats, aged 6 and over 90 days and weighing 10–15 g and 250–300 g respectively, were used. The rats were anesthetized with sodium methohexital (Brevimytal; Eli Lilly, Giessen, F.R.G.; 50 mg/kg i.p.) and bilateral microinjections were performed

stereotaxically into the rostral part of the striatum (caudate-putamen). Stereotaxic coordinates were derived from the atlas of Paxinos and Watson (27): AP 9.48, L ± 3.0, V 4.8. No rats were used for more than one pair of injections. In some animals cortical ablation (undercutting) was performed 14 days before the intrastriatal microinjections of drugs or saline. For this purpose, the animals were anesthetized with sodium methohexital and placed into the animal stereotaxic apparatus (La Precision Cinematographique; Paris, France). A hole was drilled in the parietal bone and the parietal cortex was separated from the underlying caudate nucleus unilateraly with a glass microknife. Drugs were delivered into the striatum in a volume of 1 μl at a rate of 0.1 μl/min. Aminooxyacetic acid (AOAA; Sigma, St. Louis, MO, U.S.A.) was administered into the striatum in doses of 0.1, 0.25, 0.5 and 1 μmol. Kynurenic acid (KYNA; Sigma) (0.5 μmol) or (−)-2-amino-7-phosphonoheptanoic acid (AP7; Tocris, Buckhurst Hill, Essex, U.K.) (0.25 μmol) were co-administered with AOAA (0.25 and 1 μmol) into the striatum.

For morphological examination of the brains by light microscopy 30 min - 14 days after administration of drugs or saline, rats were anesthetized with an overdose of sodium methohexital and perfused through the heart and ascending aorta with fixative containing 10% acetic acid, 10% formaldehyde, and 80% methanol. The brains were allowed to fix in situ at 4°C for 24 h and then were removed and processed for paraffin embedding. Serial coronal sections of the whole brain were cut 10 μm thick, and every 10th section was mounted on a glass slide and stained with cresyl violet or by the Fink and Heimer technique [28].

For light and electronmicroscopic examination 30 min-4 h after administration of AOAA, rats were anesthetized and perfused with fixative containing 1% paraformaldehyde and 1.5% glutaraldehyde in phosphate buffer. After perfusion for 15 min, the brains were removed, sliced in 1 mm transverse slabs, fixed in osmium tetroxide, dehydrated in graded ethanols, cleared in toluene and embedded in araldite. Sections 1 μm thick were cut with 1/2" glass knives at 40 μm intervals on a Sorval MT-2B ultratome, stained with methylene blue/azure II and evaluated by light microscopy. Ultrathin sections were cut from areas of special interest from the same blocks, stained with lead citrate and uranyl acetate, and examined with a JEOL 100B transmission electron microscope.

The activity of the enzyme L-glutamate decarboxylase (GAD) was determined in the rat striatum following injection of AOAA, AOAA + KYNA and AOAA + AP7. Striatal GAD activity in animals which received intrastriatal injection of saline served as control. Fourteen days after intrastriatal injection of drugs or saline, brains were rapidly removed and the striata dissected. GAD activity was determined spectrofluorometrically by the method of Lowe et al. [29]. After dissection the tissue was homogenized in the buffer substrate made up by combining 1 ml of 100 mM L-glutamic acid with 40 μl of 50 mM pyridoxal phosphate. GAD activity was expressed as nmol γ-aminobutyrate (GABA)/mg protein/h ± S.E.M. Protein was determined by the method of Lowry et al. (30) as modified by Markwell et al. (31). The data from biochemical experiments were evaluated by means of Student's t-test.

Results

Microinjections of AOAA (0.1-1 μmol) into the striatum of adult rats (over 90 days of age, n = 21) produced neuronal lesions in a dose-dependent manner (Fig. 1 and 2). The extent of the lesion induced by AOAA in the dose of 0.1 μmol (n = 4) was less than that induced by higher doses of the toxin (Fig. 1). The dose of 1 μmol (n = 5) induced the most pronounced deterioration of the striatal tissue. The doses of 0.25 μmol (n = 7) and 0.5 μmol (n = 5) were less toxic than 1 μmol of AOAA. Electronmicroscopic analysis revealed that the main tissue components affected were dendrites and cell somata with relative preservation of presynaptic axon terminals and glial cells (Fig. 3). Acute swelling of neuronal somata with condensation of mitochondria and disaggregation of the endo-

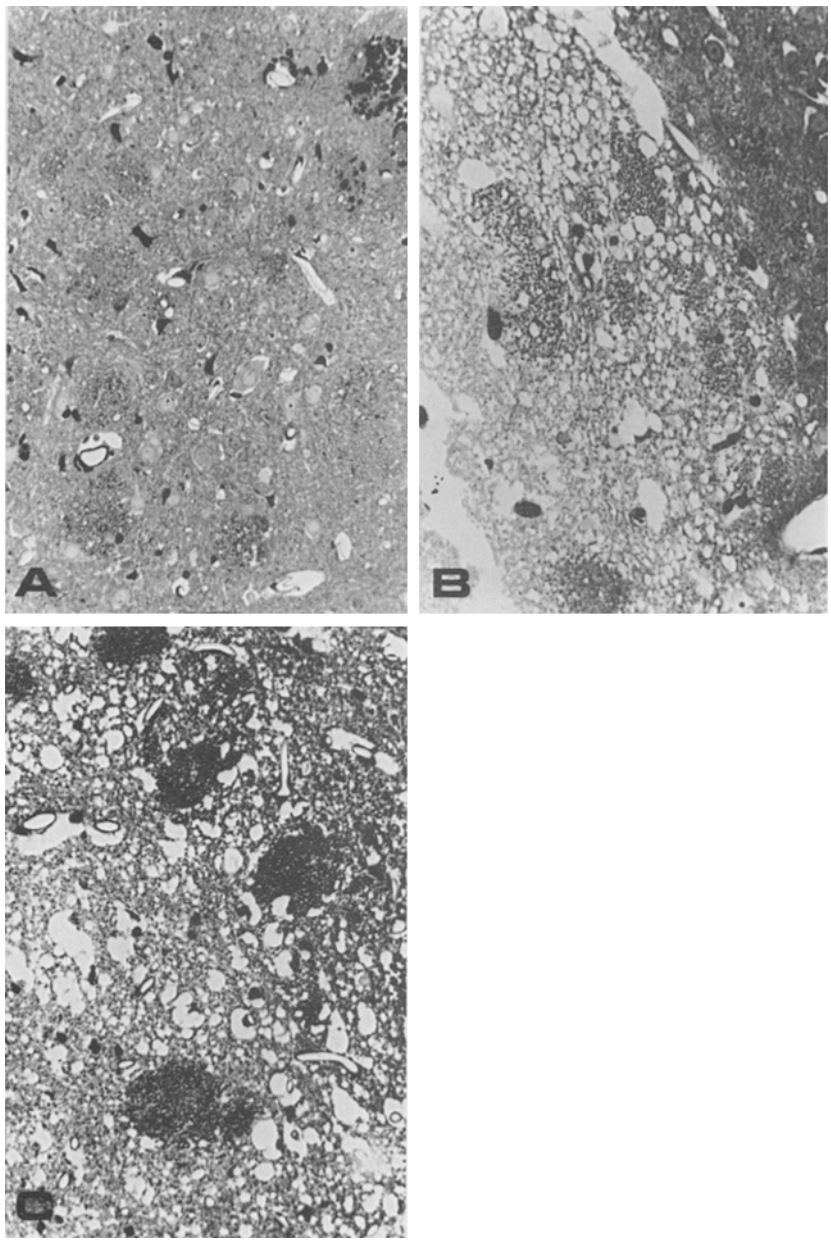

Fig. 1. Dose-relationship of neurotoxicity induced by AOAA in rat caudate-putamen. **A** Little neuronal injury is seen in the caudate-putamen of a rat subjected to microinjection of 0.1 μmol of AOAA. **B** Massive deterioration of striatal morphology observed after microinjection of 0.25 μmol of AOAA into the caudate-putamen (edge of necrotic lesion). **C** Total destruction of morphological structure of the caudate-putamen in a rat subjected to microinjection of 0.5 μmol of AOAA. Survival time: 4 h. Mag.: **A, B, C** 250x

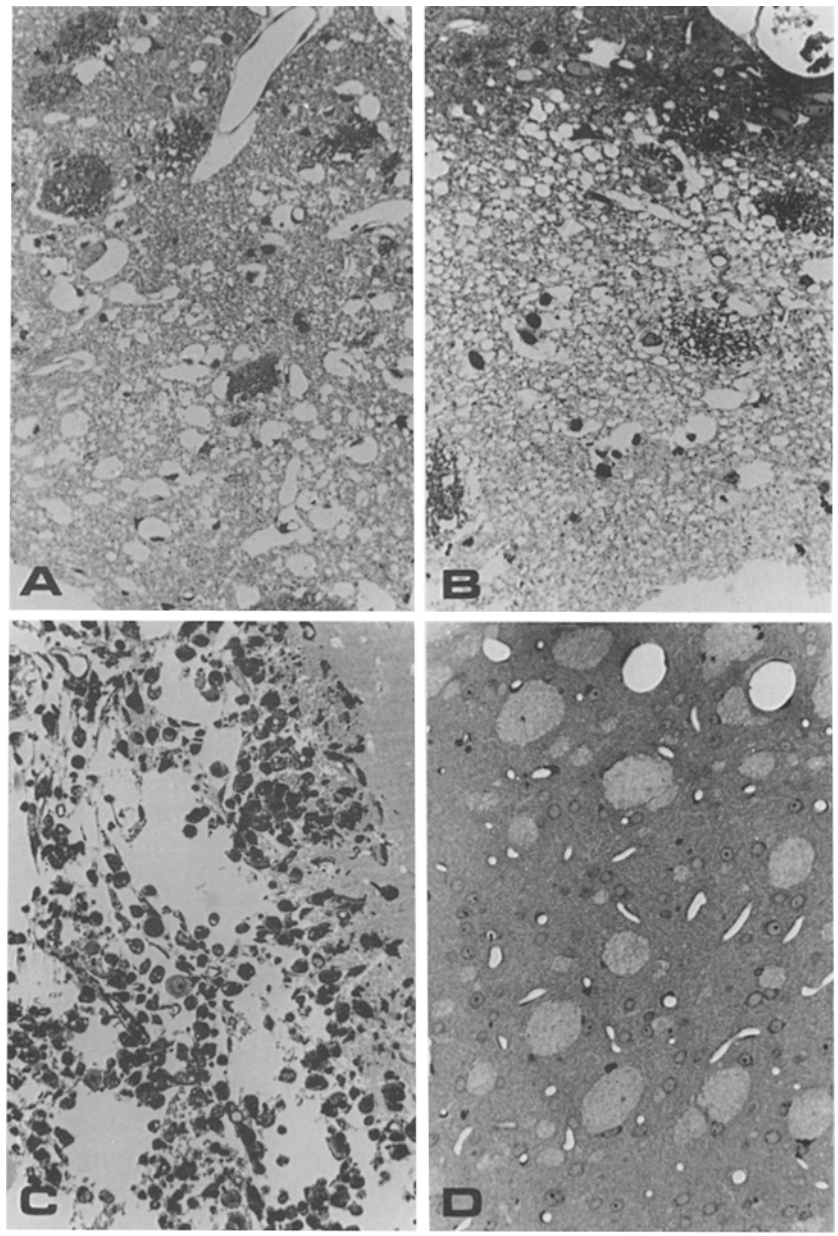

Fig. 2. Time-course of AOAA toxicity in the rat caudate-putamen. Rat caudate-putamen 30 min **(A)**, 4 h **(B, D)** and 5 days **(C)** after administration of AOAA or vehicle. There is a pronounced dilation of dendrities and vacuolation of cytoplasm in the caudate-putamen **(A)** as response to the extensive neuronal damage induced by AOAA. Neurones exhibiting vacuous swollen perikarya and pyknotic changes are apparent throughout the striatum **(A, B)**. In C marked gliosis and lack of neuronal perikarya characterize the lesioned caudate-putamen 5 days after injection of AOAA. It appears that large neurons are spared. Panel D shows the caudate-putamen of a rat which received intrastriatal injection of vehicle 4 h prior to sacrifice. There are no signs of neuronal injury in the caudate-putamen of this animal. Neuronal perikarya and axon bundles are well preserved and no glial proliferation is evident. Mag.: **A, B, C, D** 250x

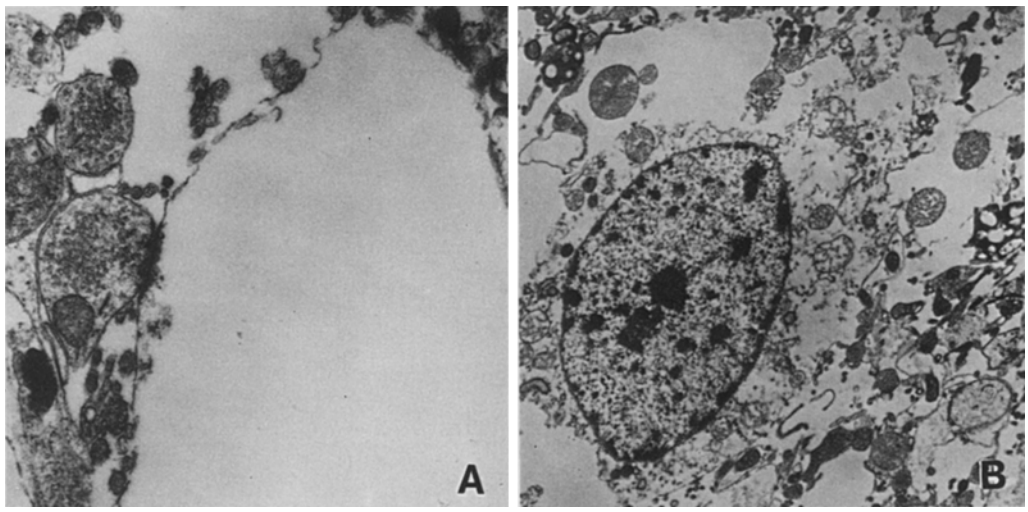

Fig. 3. Ultrastructural changes induced by AOAA in rat caudate-putamen. **A** Swollen dendrite undergoing edematous degeneration in the caudate-putamen of an adult rat which received intrastriatal microinjection of 0.25 μmol AOAA 30 min prior to sacrifice. This degenerating dendrite is in contact with a presynaptic axon terminal which appears normal. **B** Neuron undergoing edematous degeneration in the caudate-putamen of an adult rat which received intrastriatal microinjection of 0.25 μmol AOAA 30 min prior to sacrifice. Typical features of AOAA induced neuronal degeneration are the vacuolated endoplasmic reticulum, condensed mitochondria and clumped nuclear chromatin. Note that degeneration of dendrosomatic elements with relative preservation of presynaptic axon terminals characterize lesions induced by excitotoxins like glutamate (1, 2)

plasmic reticulum, were observed within 30 min after the intrastriatal injection of 0.25 μmol of AOAA (Fig. 3). After longer survival periods (1–14 days) absence of neuronal elements and profound glial proliferation characterized the lesioned striatum (Fig. 2). No signs of neuronal degeneration were seen in regions other than the injected striatum. Coadministration of AP7 (0.25 μmol, n = 8) or KYNA (0.5 μmol, n = 7) with AOAA (0.25 μmol) into the striatum of the adult rats prevented or markedly reduced neurodegeneration. In animals with unilateral ablation of the parietal neocortex (n = 10), neurodegenerative reaction to AOAA (0.25 μmol) was absent in the striatum of the decorticated side but present and unchanged in the contralateral, unlesioned side.

Intrastriatal microinjections of AOAA (0.25, 0.5 and 1 μmol) produced reduction of striatal GAD-activity as measured after a survival period of 14 days. The dose of 0.25 μmol AOAA reduced GAD-activity in lesioned striata by 18%, the dose of 0.5 μmol by 43%, and the dose of 1 μmol by 50% as compared to values of striatal GAD activity in animals which received intrastriatal injection of saline (Table 1). Coadministration of either KYNA (0.5 μmol) or AP7 (0.25 μmol) with AOAA (0.25 μmol) prevented reduction of GAD activity induced by AOAA (Table 1).

When AOAA (1 μmol) was administered into the striatum of young rats (6 days of age, n = 10) no signs of neuronal injury were observed.

Table 1. Striatal GAD-activity 14 days after intrastriatal microinjection of drugs or saline

a: Dose-dependency of AOAA-induced reduction in striatal GAD-activity	nmol GABA/mg protein/h	N
Control	110.1 ± 6.5	10
AOAA 0.25 μmol	83.6 ± 6.1*	9
AOAA 0.5 μmol	62.8 ± 4.4***	10
AOAA 1 μmol	55.9 ± 3.6***	10
b: Antagonism of AOAA-induced reduction in striatal GAD-activity by AP7	**nmol GABA/mg protein/h**	**N**
Control	116.5 ± 6.5	9
AP7 0.25 μmol	111.4 ± 5.9	10
AOAA 0.25 μmol	86.6 ± 3.0**	10
AP7 + AOAA	124.7 ± 7.2^	10
c: Antagonism of AOAA-induced reduction in striatal GAD-activity by KYNA	**nmol GABA/mg protein/h**	**N**
Control	100.6 ± 6.9	10
KYNA 0.5 μmol	84.8 ± 3.3	10
AOAA 0.25 μmol	64.1 ± 5.4**	10
KYNA + AOAA	84.1 ± 3.7^	10

Striatal GAD-activity was determined in adult rats by methods described in the text. GAD-activity is expressed as nmol GABA/ mg protein/h. The data are presented as means ± S.E.M. N represents the number of animals in experimental groups. *$P < 0.05$, **$P < 0.01$, ***$P <$ 0.001 0.001 vs vehicle treated rats; ^$P < 0.05$ vs AOAA treated rats (Student's t-test). *AOAA* aminooxyacetic acid; *KYNA* kynurenic acid.

Discussion

Our findings demonstrate that AOAA is a potent and rapidly acting neurotoxin in the adult rat brain but lacks neurotoxicity in the immature brain. Lesions produced by AOAA exhibit excitotoxic neuropathological characteristics, i.e. selectivity for dendrosomatic neuronal elements with relative preservation of presynaptic axon terminals and glial cells [1, 2]. The NMDA subtype of L-glutamate receptors is involved in the pathogenesis of AOAA-induced lesions, since AOAA-neurotoxicity can be blocked by the NMDA receptor antagonists AP7 and KYNA.

AOAA though is not an NMDA receptor agonist, since it fails to damage the immature rat CNS which reportedly is extremely prone to NMDA [32, 33]. Furthermore, AOAA neurotoxicity in the striatum is dependent upon the presence of intact corticostriatal afferents, is restricted around the injection site and

fails to produce distant cell loss. These neurotoxic properties of AOAA are identical to those of QUIN [11, 12].

Since AOAA is an inhibitor of the synthesis of KYNA, an endogenous antagonist at L-glutamate receptors [9], one may suggest that blockade of its neuroprotective effect is responsible for the observed excitotoxic reaction. Deficient neuroprotection by endogenous L-glutamate antagonists, such as KYNA, is a mechanism which might be involved in the pathogenesis of human neurodegenerative disorders. Huntington's disease, an autosomal dominant neurological disorder which starts in midlife, shows neuropathological characteristics which can be reproduced by QUIN (9). Attempts to verify an increase of QUIN concentrations in the brains of Huntington's disease victims have been unsuccessful so far. It might be interesting to test, whether hypofunction of the endogenous KYNA system might be responsible for neuronal degeneration in Huntington's disease.

In in vitro preparations, the endogenous L-glutamate can trigger excitotoxic cell death if intracellular energy metabolism is compromised [7, 23, 34]. This toxic effect may be due to alterations in cell membrane function leading to partial depolarization and hence to a relief of the voltage-dependent Mg^{2+} block of NMDA receptor channels [7]. Chemically-induced hypoglycemia [23], potassium cyanide [23], or potassium-induced depolarization [34] in chick retina can produce excitotoxic lesions. Such lesions are not accompanied by enhanced L-glutamate release and can be blocked by NMDA antagonists [23]. AOAA may interfere with intracellular energy metabolism in neurons [26]. Thus, according to this hypothesis, AOAA may increase the sensitivity of neurons to EAA toxicity, allowing even normally available synaptic L-glutamate concentrations to induce a neurotoxic effect. It is important to note that a mitochondrial complex I deficiency has been demonstrated in patients with Huntington's disease [35]. A regionally selective mitochondrial complex I deficiency has been already demonstrated in the substantia nigra of patients with Parkinson's disease [25]. There are also some apparent similarities between neurotoxic profiles of AOAA and MPP^+ in the rodent brain [24]. Such observations raise the possibility that regional impairment in energy metabolism may secondary result in excitotoxic tissue reactions and perhaps in neurodegenerative processes, such as in Huntington's disease.

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Authors' address: Dr. W. A. Turski, Department of Pharmacology, Medical School, Jaczewskiego 8, PL-20-090 Lublin, Poland.

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