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EFFECT OF PESTICIDES ON KYNURENIC ACID PRODUCTION IN RAT BRAIN SLICES

Elżbieta Zielińska[1], Tomasz Kocki[2], Tomasz Saran[1], Sandor Borbely[3], Damian Kuc[2], Ildiko Vilagi[3], Ewa M. Urbańska[1, 2], Waldemar A. Turski[1, 2]

[1]Department of Toxicology, Institute of Agricultural Medicine, Lublin, Poland
[2]Department of Pharmacology and Toxicology, Medical University, Lublin, Poland
[3]Department of Physiology and Neurobiology, Eotvos Lorand University, Budapest, Hungary

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Abstract: Kynurenic acid (KYNA) is a broad spectrum antagonist of ionotropic glutamate receptors, preferentially active at the strychnine-insensitive glycine allosteric site of the N-methyl-D-aspartate (NMDA) receptor, and a noncompetitive antagonist of alpha7 nicotinic receptor. Animal studies showed that it possesses anticonvulsant and neuroprotective properties. Its involvement in the pathophysiology of various brain disorders was suggested. In this study, the effect of pesticides on KYNA production in brain cortical slices was investigated. Pyrethroids, deltamethrin and fenpropathrin significantly lowered KYNA production. Methomyl, bensultap, fipronil, diquat and MCPA were ineffective in this regard. In view of this data, the inhibition of KYNA synthesis appear to merit further investigation as a potential factor contributing to the toxicology of pyrethroids.

Address for correspondence: Waldemar Turski, Department of Pharmacology and Toxicology, Medical University, Jaczewskiego 8, 20-090 Lublin, Poland.
E-mail: waldemar.turski@am.lublin.pl

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INTRODUCTION

Kynurenic acid (KYNA) is a tryptophan metabolite which is synthesized from kynurenine by kynurenine aminotransferases (EC 2.6.1.7). KYNA is a broad spectrum antagonist of ionotropic glutamate receptors [18], preferentially blocking the strychnine-insensitive glycine allosteric site of the N-methyl-D-aspartate (NMDA) receptor [3]. It has been also demonstrated that KYNA is a non-competitive antagonist of alpha7 nicotinic receptor [9].

Animal studies have shown that KYNA possesses anticonvulsant and neuroprotective properties [7, 14]. It has been suggested as being involved in the pathophysiology of several brain disorders, e.g. Parkinson's disease [17], Huntington's disease [1, 2] and epilepsy [28], and might be causally related to the pathology of excitotoxic retinal diseases [23].

Annually, millions of tons of pesticides are applied to control pest organisms. Most of this application is targeted on agricultural crops. However, they are widely used also on forests, parks, roadsides, homes, offices and schools. This widespread use of pesticides pollutes our environment and threatens public health. While pesticides are designed to kill specific plants or insects, they often have harmful effects on non-target species, including humans. The mammalian nervous system seems to be an especially sensitive target for pesticide action.

Here, we investigate the effect exerted by pesticides upon KYNA synthesis in rat cortical brain slices.

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MATERIALS AND METHODS

KYNA production in cortical slices. The experiments were carried out on adult male Wistar rats obtained from a licensed supplier. Experimental procedures were approved by the Local Ethical Committee in Lublin, Poland.

Rat cortical slices (1×1 mm base) obtained from the same animal were placed randomly in culture wells (8 per well) containing the oxygenated Krebs-Ringer buffer (pH 7.4). Slices were subjected to tested pesticide and 10 μM L-kynurenine and incubated at 37ºC for 2 hr. At the end of incubation, supernatants were collected, 50% trichloroacetic acid was added and precipitated protein was removed by centrifugation. Supernatants were applied to cation-exchange columns (Dowex 50 W^+). Eluate was subjected to high performance liquid chromatography (HPLC) (C_{18} reverse-phase column) and KYNA was detected fluorimetrically (excitation: 334 nm; emission: 398 nm, detection limit: 0.1 pmol), as described previously [25, 26]. The mean control production of KYNA was 6.0 ± 0.5 pmol/h per well.

Substances. (4-Chloro-2-methylphenoxy) acetic acid (MCPA), deltamethrin, fenpropathrin and methomyl were purchased from the Institute of Organic Industrial Chemistry (Poland). Bensultap and diquat were obtained from Dr Ehrenstorfer GmbH (Germany) and fipronil from Rhône-Poulenc (France). KYNA and L-kynurenine sulfate were purchased from Sigma (USA). HPLC reagents were obtained from Baker. All other substances were of the highest available purity.

Data analysis. Statistical analysis of data was performed using Student's *t*-test.

RESULTS

Deltamethrin and fenpropathrin at concentration of 0.1 mM significantly lowered KYNA production by 31.1% and 31.7%, respectively. KYNA production was not affected by bensultap and fipronil at concentrations up to 0.1 mM and diquat, MCPA and methomyl at concentrations up to 1 mM (Tab. 1).

Table 1. Effect of pesticides on kynurenic acid (KYNA) production in brain cortical slices. Results are expressed as percentage of the respective control; mean ± SEM; n = 6 experiments (p - probability, NS - not significantly different).

Treatment [mM]	KYNA production [%]	p
Deltamethrin 0.1	60.9 ± 6.5	<0.05
Fenpropathrin 0.1	60.3 ± 4.5	<0.05
Bensultap 0.1	105.4 ± 7.7	NS
Fipronil 0.1	93.3 ± 8.3	NS
Diquat 1.0	92.7 ± 3.6	NS
MCPA 1.0	109.6 ± 8.4	NS
Methomyl 1.0	99.4 ± 5.2	NS

DISCUSSION

We found that pyrethroids, deltamethrin and fenpropathrin significantly diminished KYNA production in rat cortical slices. Other tested insecticides which interact with important brain neurotransmitter mechanisms, methomyl - a carbamate acetylcholinesterase inhibitor, bensultap - a nicotinic receptor channel blocker and fipronil - a GABA antagonist or herbicides which do not interfere with neurotransmission, diquat and MCPA were ineffective in this respect.

Pyrethroids modulate sodium channel activity in excitable tissue and the most characteristic feature of their action is the occurrence of a large and prolonged tail current on termination of depolarizing pulse [15]. Since it was found that depolarising concentration of K^+ lowered KYNA synthesis in brain slices under similar conditions [27], it can be speculated that depolarisation due to change of sodium channels permeability is responsible for the inhibition of KYNA synthesis exerted by pyrethroids.

Nowadays a new generation of insecticides is being developed. Fipronil acts on insect GABA receptors, which are similar to the mammalian ligand gated $GABA_A$ or $GABA_C$ receptors [5, 10]. Fipronil binds to a special allosteric binding site [21, 22, 23] and functions as GABA antagonist. It inhibits chloride channels, so it might have a convulsive effect. However, in the concentration investigated, which has proper insecticide potency, it showed no effect on the KYNA synthesis.

The nereistoxin analogue bensultap [13, 16] inhibits nicotinic ACh receptors, the structure of which is also similar to the mammalian nACh receptors [4, 11]. In mammals, these receptors usually are located at the presynaptic membranes and regulate the transmitter release. Although bensultap can pass the blood-brain barrier [6], it has only a transient effect on neuronal activity (personal communication Dr. I. Vilagi) and did not affect KYNA synthesis in our experimental paradigm.

KYNA is a broad-spectrum antagonist of ionotropic glutamate receptors, preferentially blocking the glycine allosteric site of the NMDA receptor, and a noncompetitive antagonist of alpha7 nicotinic receptor [3, 9, 18]. It has been suggested that the reduced concentration of KYNA in the brain due to the inhibition of its synthesis may cause seizures and neurodegeneration in rodents [14, 24, 27].

In humans, acute intoxication with pyrethroids results in several neurological symptoms. He *et al.*, (1989) analysed 573 cases of acute pyrethroid poisoning and recorded dizziness in 60 per cent, headache in 45%, fatigue in 26% and blurred vision in 7% of patients. Convulsions were also observed. They complicated severe acute pyrethroid poisonings and were stated as a cause of death in 4 of 7 fatalities [8].

Numerous epidemiological studies showed that the long-term occupational exposure to pesticides may be associated with parkinsonism, although no direct connection

with specific pesticides was definitely proved (see for review [12]). On the other hand, experiments conducted on laboratory animals revealed that exposure to pyrethroids may produce damage or functional changes within the dopaminergic nigrostriatal pathway which is the primary neurodegenerative substrate of Parkinson's disease [19].

Interestingly, in brain tissue and cerebrospinal fluid in Parkinson's disease decreased concentration of KYNA was detected [17]. Moreover, a reduced formation of KYNA was reported in Huntington's disease patients [1, 2].

CONCLUSION

It should be stressed that at present pyrethroids are being used widely as almost ideal insecticides due to their high insecticidal potency and low mammalian toxicity [15]. Thus, in view of the present data, the inhibition of KYNA synthesis appear to merit further investigation as a potential factor contributing to the toxicology of pyrethroids.

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