BIOLOGICAL ACTIVITY OF TREMORGENIC MYKOTOXINS

M. WEISER, J. FINK-GREMMELS and L. LEISTNER

Institute for Microbiology, Toxicology and Histology

Federal Centre for Meat Research, 8650 Kulmbach (Federal Republic of Germany)

INTRODUCTION: Tremorgenic mycotoxins are secondary metabolites and they are specific in their biological activity. Clinical signs are tremor, akinesia, rigor and ataxia. Cyclopiazonic acid, penitrem A and verrucosidin are considered to be tremorgenic neurotoxins (ref. 1,2,3). Signs of this toxicity are similar to symptoms typically for Parkinson's disease, which is perhaps related to a reduction of dopaminergic activity in the substantia nigra. Tremorgens assert their activity by modulating the release of neurotransmitters in the central nervous system (ref. 4,5).

The presented pharmacological and ethological experiments were designed to investigate the dose dependent sensitivity (tremorgenic dosage, tremor onset and duration) of mice to these toxins. Studies on behaviour and reactivity of mice after injection of tremorgenic mycotoxins together with pharmacological active compounds as well as evaluation of the reversibility of neurotoxic symptoms in relation to time are described.

MATERIAL and METHODS: Animals (NMRI-mice) were housed under standard conditions with free access to food and water. 1. Mycotoxins were administered in increasing dosages (dissolved in DMSO, i.p.): penitrem A (0.1/0.15/0.25/0.5/1/1.5/2 mg/kg b.w.), cyclopiazonic acid (2/5/10/15/20 mg/kg b.w.) and verrucosidin (1/3/5/10/20 mg/kg b.w.). Onset, duration, tremor incidence and mortality were recorded (mean effective and mean lethal dose were calculated). 2. Alterations in tremor response by various pharmacological active compounds injected in increasing dosages 30 minutes prior to penitrem A were examined. Compound utilized were atropine, amantadine, bromocriptine, chlorpromazine, L-dopa, mephensine and reserpine. 3. Ethological investigations: a) Learning behaviour was determined in the dark-avoidance-test, using the inborn negative phototactic behaviour. b) Spontaneous motility was recorded after penitrem A administration. c) Alterations of behaviour were investigated by using the IRWIN-Test.

RESULTS: Mean lethal dosage for cyclopiazonic acid was 9.93 (6.39-15.45) mg/kg b.w. and for verrucosidin 4.37 (2.90-6.58) mg/kg b.w.. Both mycotoxins proved to be strong neurotoxic compounds, but clinical signs of intoxication differed from those of penitrem A. Sublethal dosages caused incoordination, sedation, muscle relaxation, but no tremor in mice. The animals recovered completely within a few hours. Therefore no further tests were performed with these mycotoxins.

Penitrem A induced a spontaneous, but reversible tremor lasting 2-3 hours after injection.

tion. Mean effective dosage was 0.22 (0.11-0.42) mg/kg b.w.. Mean lethal dosage was 0.96 (0.51-1.81) mg/kg. In concentrations exceeding 1 mg/kg b.w. convulsions were observed overshadowing the tremorgenic symptoms. Onset of a penitrem A-induced tremor could not be suppressed in physiological dosages with the tested pharmacological active compounds. Nevertheless all of these influenced in different degrees the tremor onset and duration (Tab. 1). Mephenesine reduced dose dependent tremor incidence. The ethological tests did not prove an anamnestic effect of penitrem A, but motility was significantly reduced during tremor. The distinct alterations of behaviour investigated in the IRWIN-Test are shown in Tab. 2.

Tab. 1 Influence of pharmacological active compounds on Penitrem A-induced tremor onset and duration

compound	Tremor onset	Tremor duration	
Amantadine	--	+	
Atropine	+	-	
Bromocriptine	+	(-)	+ = increase
Chlorpromazine	+	-	- = decrease
L-dopa	(+)	(-)	
Mephenesine	++	--	
Reserpine	(+)	++	

Tab. 2 Results of the IRWIN-Test

Toxin	Concentration (mg/kg b.w.)	Score (x $\pm$ SD, number)
Control group	2.0 μ l DMSO/g b.w.	55.2 $\pm$ 0.8 , 6
Cyclopiazonic acid	7.7	49.0 $\pm$ 3.6 *, 6
Penitrem A	0.22	42.9 $\pm$ 1.7 ***, 6
Verrucosidin	3.0	40.7 $\pm$ 2.7 ***, 6

CONCLUSIONS: Penitrem A induces symptoms which are correlated to Parkinson's disease. The reported experiments gave no clear evidences for an involvement either of the cholinergic or catecholaminergic neurotransmitters in penitrem A neurotoxicity (ref. 5). Therefore an interaction with inhibitory or excitatory amino acids in the CNS has to be considered (ref. 4). For the toxicological significance it is important that neurotoxicity is spontaneously reversible within a few hours in mice (ref. 2).

Cyclopiazonic acid and verrucosidin are reported to be tremorgens (ref. 1,3). Our experiments demonstrated that both mycotoxins are neurotoxic compounds, but clinical symptoms of intoxication differ from those of other tremorgens and thus they appear to represent subclasses of tremorgens. Therefore, investigations of the molecular activity of tremorgenic mycotoxins are necessary.

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