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MK-801 antagonizes the lethal action of centrally and peripherally administered cypermethrin in mice and rats

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Abstract—The present study investigated the effect of MK-801, an *N*-methyl-D-aspartate antagonist, on the convulsant lethal action of cypermethrin administered centrally or peripherally. Cypermethrin produced severe convulsions and death in a dose-dependent manner. MK-801 (0·5, 1 and 2 mg kg^{-1}, intraperitoneally) significantly increased the onset time of convulsions and decreased the mortality in the peripherally treated cypermethrin group. MK-801 (1·0 and 2·0 mg kg^{-1}) attenuated the convulsant action of cypermethrin (50 μg, intracerebroventricularly) significantly. Survival rate was also increased significantly. However, MK-801 (0·5 mg kg^{-1}) did not produce any significant protective effect against centrally administered cypermethrin. These results suggest excitatory amino acids to be a target for pyrethroid-induced neurotoxicity.

Recent years have seen major progress in the understanding of excitatory amino acid synaptic mechanisms and their involvement in brain function and disease (Fagg et al 1986). The excitatory amino acids, L-glutamate and L-aspartate, exert their physiological and pathophysiological effects via distinct receptor subtypes that have been characterized in terms of preferred agonists (Watkin 1984). *N*-Methyl-D-aspartate (NMDA) receptors are the most well characterized of these receptor subtypes and have been implicated in pathophysiological mechanisms underlying such phenomena as epileptic discharges, anxiety, spasticity, and ischaemic and hypoglycaemic neuronal discharge (Kemp et al 1987). Thus, compounds that act as NMDA receptor antagonists could have important therapeutic uses (Meldrum 1985). 2-Amino-5-phosphonopentanoate (AP_5) or 2-amino-7-phosphonoheptanoate (AP_7), the selective NMDA antagonists, have been shown to exert anticonvulsant actions in different seizure models (Loscher 1985), and to protect from ischaemic and hypoglycaemic brain damage (Meldrum 1985).

The last decade has seen the development of new photostable and highly effective insecticidal pyrethroids, that are neurotoxic to mammals and insects (Ramadan et al 1988a). The major toxic signs of type II pyrethroids have been characterized by a rapid onset of tremors, profuse salivation, abdominal constriction and tonic-clonic convulsions (Ramadan et al 1988a). Most of these signs are produced when pyrethroids are injected into the central nervous system (Gray 1985). NMDA antagonists have already been shown to be effective as anti-convulsants, and there have been, to our knowledge, no in-vivo report that shows the involvement of excitatory amino acids in the toxicity of type II pyrethroids. Hence, we have investigated the effect of MK-801 on the convulsive action of cypermethrin injected centrally. We have also studied the effect of MK-801 on peripherally administered cypermethrin in mice.

Materials and methods

The study was performed in male ICRC Swiss albino mice, 20–25 g, and Wistar rats, 150–200 g, obtained from the Central Animal House of the Institute. All animals were allowed a 2-week acclimatization period before experimentation. The animals were housed in a room with constant temperature (23 ± 2°C), controlled humidity (60 ± 10%) and were exposed to a 12 h light/dark cycle. Food and water were freely available in the home cage. All the convulsive experiments were performed in a dimly-lit, sound-proof room.

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Study design. To study the effect of MK-801 on peripherally administered cypermethrin, the animals were divided into four groups. All animals were given cypermethrin (60 mg kg^{-1}, intraperitoneally) and subsequently put into individual perspex cages (25 × 25 × 10 cm). The animals were observed for general behaviour and occurrence of tonic-clonic convulsions for 60 min. The convulsive episode was adjudged to occur when there were severe myoclonic jerks followed by tonic convulsions. The mortality was recorded for 24 h after the dose. The dose of cypermethrin was selected from our preliminary studies and was sufficient to produce convulsions and death in 100% of the animals. Fifteen min later, three groups were treated with MK-801 (0·5, 1 and 2 mg kg^{-1}, intraperitoneally) while the fourth group received the vehicle and served as untreated control. The parameters recorded were: onset time of frank tonic-clonic seizures and percent animals dead.

For intracerebroventricular studies, Wistar rats were used. Cypermethrin in graded doses was injected into the right lateral cerebral ventricle through a permanent indwelling cannula fixed on the skull as described by Noble et al (1964). Injections were made with the help of a Hamilton microsyringe and the volume of the injection was kept constant (20 μL/rat). To study the effect of MK-801 (0·5, 1 and 2 mg kg^{-1}) cypermethrin was injected at a dose of 50 μg/rat; MK-801 was administered peripherally 15 min before the administration of cypermethrin.

The parameters recorded were: time of onset of tonic-clonic seizures, survival time and survival rate.

Drugs. MK-801 ((+)-5-methyl-10,11-dihydro-5*H*-dibenzo-le[*a*,*d*]cyclohepten-5,10-imine maleate, Merck Sharp & Dohme, UK) was dissolved in distilled water and adjusted to a neutral pH (7·3 ± 0·1) with NaOH. Cypermethrin was diluted with simulated cerebrospinal fluid (NaCl 128 nM, KCl 2·5 mM, $MgCl_2$ 2·0 mM, $CaCl_2$ 1–3 mM). Control animals received an equivalent volume of simulated cerebrospinal fluid. All drug solutions were prepared just before the experimentation.

Statistics. To compare the difference between the mean values of time of onset of convulsions and time of death, Student's unpaired *t*-test was used. The difference between the mortality rate was evaluated by Chi square test with Yate's correction. $P < 0.05$ was considered significant.

Results

Cypermethrin produced severe tonic-clonic convulsions when administered centrally as well as peripherally. At 60 mg kg^{-1}, cypermethrin produced highly reproducible convulsions and lethality. All the mice injected with 60 mg kg^{-1} of cypermethrin showed tonic-clonic convulsions and died after the episode of frank convulsions. Mice showed increased respiration, tremors, incoordination, abdominal constriction, hyperexcitability and tail erection before the onset of convulsions.

A graded dose-response effect on the episodes of convulsion

Table 1. Effect of MK-801 on convulsions induced by the intraperitoneal injection of cypermethrin when administered peripherally to mice (n = 8).

Treatment (mg kg^{-1})	Time of onset (min) (mean ± s.d.)	Deaths (%)
Cypermethrin (60)	45 ± 7	100
+ MK-801 (0·5)	54 ± 6·2*	62
+ MK-801 (1·0)	60 ± 9·5*	37*
+ MK-801 (2·0)	68 ± 7·4*	0*

* $P < 0.05$ compared with cypermethrin alone.

Table 2. Effect of MK-801 on convulsions induced by the intracerebroventricular injection of cypermethrin when administered peripherally to rats (n = 8).

Treatment				
Cypermethrin (μg)	MK-801 (mg kg^{-1})	Time of onset (min) (mean ± s.d.)	Time to death (min) (mean ± s.d.)	Mortality
12·5	—	28 ± 2·1	49 ± 7	3/8
25	—	25 ± 3·3	45 ± 5·7	5/8
50	—	21 ± 2·3	35 ± 7	8/8
50	0·5	24 ± 4·1	33 ± 6	6/8
50	1·0	28 ± 4·6*	50 ± 12*	2/8*
50	2·0	35 ± 3·9*	0 ± 0*	0/8*

*$P < 0.05$ compared with cypermethrin (50 μg) alone.

and post-convulsive lethality was observed when cypermethrin was injected centrally. Severe tremors, hyperexcitability and increased respiration occurred before the onset of convulsions.

Table 1 shows the effect of MK-801 on cypermethrin-induced convulsions injected peripherally. MK-801 increased the onset time of convulsions in a dose-dependent manner. MK-801 reduced the severity of convulsions while it completely blocked tremors, increased respiration, abdominal constriction and hyperexcitability. At 2 mg kg^{-1}, MK-801 completely protected the animals against the lethal action of cypermethrin. Table 2 shows the results of MK-801 (0·5, 1, 2 mg kg^{-1}) against centrally injected cypermethrin. At 1 and 2 mg kg^{-1}, MK-801 significantly delayed the onset time of convulsions and time to death. A complete protection against lethal action of cypermethrin was seen at 2 mg kg^{-1} MK-801. All the other behavioural signs were completely blocked with MK-801.

Discussion

Synthetic pyrethroids have emerged as a major class of insecticides due to their high potency and selectivity as nerve poisons (Casida et al 1983). Research into mechanisms of action of pyrethroid has shown that they interact with neuronal sodium channels to produce prolongation of the sodium current (Lund & Narahashi 1983). The type II pyrethroids, however, appear to have additional actions at the $GABA_A$ receptor/Cl ionophore (Crofton et al 1987). Recent evidence also implicates the peripheral type benzodiazepine receptor as a site of action for type II pyrethroids (Ramadan et al 1988b). Type II pyrethroids have been shown to affect cholinergic and noradrenergic transmission in producing their behavioural effects (Chugh et al 1991).

The results of the present investigation have demonstrated that cypermethrin is a potent proconvulsant in mice and rats. These results also demonstrate that there is more than one site or mode of action of pyrethroids. Type II pyrethroids have been shown to act as GABA antagonists (Nutt et al 1982). Glutamate or other synaptic sites are closely associated with GABA receptors and have been postulated as the possible targets for type II pyrethroids (Gammon et al 1981). It is probable that the antagonism of GABA by cypermethrin might lead to overactivity of excitatory amino acids, resulting in convulsions. Morimoto (1989) has suggested that a collapse of GABA mediated inhibition and activation of NMDA receptors could be an essential seizure-triggering mechanism. Deltamethrin, another type II pyrethroid has been shown to inhibit the binding of kainic acid to mouse forebrain membranes (Staatz et al 1981). These observations suggest that excitatory amino acid receptors might be a possible target for the convulsive action of cypermethrin. This is supported by the fact that MK-801 antagonized the convulsant action of both peripherally and centrally administered cypermethrin. MK-801 has been shown to interact with various other neurotransmitter receptors such as 5-HT_3 and $GABA_A$. It also has an action on the nicotinic channel complex (Halliwell et al 1989). However, the implications of these interactions is still unclear. To our knowledge, no information is available to show the involvement of nicotinic or 5-HT_3 receptors in the lethal action of cypermethrin. Therefore the most probable action of cypermethrin seems to be $GABA_A$ antagonism leading to overactivity of excitatory amino acids, which is antagonized by MK-801. MK-801 at the doses used in this study produced severe ataxia in all the animals. This response, however, did not interefere with the occurrence of convulsions.

In conclusion, our results have demonstrated in-vivo, for the first time, that the NMDA antagonist MK-801 antagonizes the convulsant action of cypermethrin. These results also suggest that an NMDA-receptor antagonist might prove to be effective in attenuating the acute toxicity of synthetic type II pyrethroids.

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Effect of ryanodine on histamine release from rat peritoneal mast cells induced by anti-IgE

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Abstract—Ryanodine strongly inhibited histamine release from rat peritoneal mast cells induced by anti-IgE. Ryanodine also inhibited Ca^{2+}-mobilization from the intracellular Ca^{2+}-store as well as histamine release in mast cells activated by anti-IgE. These results suggest that the effect of ryanodine on histamine release from rat mast cells might be due to the inhibition of Ca^{2+} release from the intracellular Ca^{2+} store.

Ca^{2+} acts as a second messenger during cell activation (Rasmman & Goodman 1977). An increase in the intracellular Ca^{2+} level has been proposed as an essential trigger for mast cell activation. Many workers have demonstrated an absolute requirement for calcium in histamine release induced by a variety of secretagogues. Histamine release from mast cells has been shown to be preceded by an increased mobilization of intracellular Ca^{2+} (White et al 1985). We have previously shown that the mobilization of calcium from the intracellular Ca^{2+} store is obligatory for histamine release induced by various secretagogues (Takei et al 1989, 1991).

Ryanodine, an alkaloid from *Ryania speciosa* Vahl, acts specifically on the Ca^{2+} release channel of the sarcoplasmic reticulum (SR) (Alexandre 1985). Ryanodine also diminishes the rate of Ca^{2+} accumulation into the SR.

We have investigated the effects of ryanodine on the change in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and on the histamine release, induced by the secretagogues, anti-IgE, concanavalin A or compound 48/80, from rat mast cells.

Materials and methods

Rats were killed by asphyxiation in an atmosphere of CO_2. Rat peritoneal mast cells were obtained from male Wistar rats, 200–300 g, and mast cells were purified using the method of Németh & Röhlich (1980). Viability of the cells was >97% as assessed by trypan blue occlusion. Passively sensitized rat mast cells were prepared as described previously (Takei et al 1988).

Assay of histamine. Purified mast cells from normal rats were incubated for passive sensitization with sensitized rat sera for 1 h at 4°C. The mast cell suspensions in Tyrode-HEPES solution (pH 7·4) were incubated in duplicate at 37°C with or without ryanodine for 5 min before the addition of secretagogues. Tyrode-HEPES solution contained (mM): NaCl 124, KCl 4·0, $CaCl_2$ 1·0, $NaHCO_3$ 10, glucose 5·6, NaH_2PO_4 0·64, $MgSO_4$ 0·5, 4-(2-hydroxyethyl)-1-piperazine-ethane sulphonic acid (HEPES) 5, and 50 mg mL^{-1} of phosphatidylserine and 50 mg L^{-1} bovine serum albumin. Bovine serum albumin was omitted from the solution in experiments on histamine release. At intervals, samples of the solution were withdrawn and the reaction was stopped by the addition of 1 mL of Tris-EDTA buffer. The Tris-EDTA buffer contained (mM): Tris 25, NaCl 120, KCl 5, EDTA 1 and 0·2 g L^{-1} human serum albumin. The cells were separated from the released histamine by centrifugation at 1300 *g* for 10 min at 4°C. Residual histamine in the cells was released by disrupting the cells with trichloroacetic acid (final concentration 10% trichloroacetic acid), and centrifugation at 1500 *g* for 15 min at 4°C. Histamine content was determined fluorometrically (Shore et al 1959). The amount of histamine released was calculated as percentage of the total histamine present in the control suspension.

Mast cells were incubated with 3 mM EGTA for 3 min at 37°C and then an optimal concentration of anti-IgE (200 μg mL^{-1}) was added to the mixture.

Measurement of intracellular calcium concentration. Fluorescence was recorded using a fluorimeter (model 650-40 Fluorescence 100; Hitachi, Japan) with a temperature-controlled cuvette and a magnetically driven stirrer. Purified mast cells (1×10^6 cells mL^{-1}) were incubated at 37°C for 10 min with 100 μM Quin-2/AM (2-[(2-amino-5-methyl-phenoxy)methyl]-6-methoxy-8-aminoquinoline-*N*,*N*,*N*′,*N*′-tetraacetic acid, tetraacetoxymethyl ester) in Tyrode-HEPES solution. The cell suspension was diluted (1 : 10) with Tyrode-HEPES solution, left to stand for 60 min, and then washed twice with the complete solution. Samples of the cell suspension (1×10^5 cells mL^{-1}) were placed in the cuvette described above, and all reagents were added with a microsyringe directly into the cuvette, without interrupting the recording. The cell suspension was incubated at 37°C for 5 min with ryanodine and challenged with the secretagogue. Fluorescence excitation and emission wavelengths

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