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Effect of cypermethrin on memory, movement activity and co-ordination in mice after transient incomplete cerebral ischemia

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Abstract:
Cypermethrin is a synthetic pyrethroid widely used as an insecticide. The aim of the present study was to investigate the possible effect of 0.1 LD_{50} of cypermethrin on memory, movement activity and co-ordination in mice exposed to transient incomplete cerebral ischemia. Transient occlusion of both carotid arteries (BCCA) in adult female mice was performed under ketamine + xylazine anesthesia. Intraperitoneal LD_{50} for cypermethrin was calculated to be 169.9 mg/kg. Memory retention was evaluated in a step-through passive avoidance task (PA), working spatial memory in a Y-maze, spontaneous movement activity in an automated device fitted with two photocells and a counter in two subsequent 30-min periods, and movement co-ordination on a rod spinning at the rate of 10 rotations/min. Neither memory nor movement co-ordination were significantly affected by transient incomplete cerebral ischemia or cypermethrin. BCCA itself did not impair movement activity in the examined mice. Cypermethrin decreased exploratory motor activity in the mice, and the effect was exacerbated by BCCA. These results show that transient incomplete cerebral ischemia combined with exposure to subtoxic doses of cypermethrin do not impair memory, but do affect behavior, producing transient reduction of spontaneous horizontal movement in mice.

Key words:
cypermethrin, transient cerebral ischemia, memory, movement activity, movement co-ordination

Introduction

Cypermethrin [(RS)-α-cyano-3-phenoxybenzyl(1RS)-*cis-trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate] is a photo-stable synthetic pyrethroid with great insecticidal potency. Inclusion of an α-cyano substituent in the phenoxybenzyl alcohol moiety, allow cypermethrin to be classified as type II pyrethroid [36]. The primary action of cypermethrin in animals is on the central nervous system [30]. Interaction of cypermethrin with sodium channels produces a hyperexcitable state and is the major mechanism of its neurotoxicity [30] and its high insecticidal activity. Mammalians are relatively resistant to the toxic actions of cypermethrin and other pyrethroids due to their fast metabolic rate, high internal body temperature and low sensitivity of target sites relative to insects [38]. Insect sodium channels are 100-fold more sensitive to cypermethrin than tetrodotoxin-resistant sodium channels in developing mammalian brains and adult dorsal root ganglia [37, 42].

Type II acute pyrethroid poisoning *via* oral or intravenous route in rats manifests itself with salivation, nosing, increased jaw opening, rolling gait due to increased extensor tone in the hind limbs, movement in-

coordination and tremor, followed by choreoatetosis, tonic seizures, apnea, and death [31]. Human type II acute pyrethroid poisoning *via* the dermal route is characterised by paresthesia, dizziness, nausea and muscular fasciculations [7] and salivation, pulmonary edema, seizures and coma *via* the oral route [10].

There are data that exposure to type II pyrethroids produces memory deficits in rats [22].

The average life span in humans is on the rise [17], and health needs of the growing older population are drawing much attention. Transient ischemic attacks (TIAs) caused by narrowing or occlusion of carotid arteries are common health problems in elderly people, and brain ischemia induces neuronal damage [9]. According to recent data, brain ischemia, even transient, produces chronic disruption of blood-brain barrier (BBB) and irreversibly damages neurons in areas engaged in memory processes and movement control [27, 43]. Brain ischemia produces chronic disturbances in protein expression [33] and injury of BBB that allows β-amyloid to enter the brain tissue and produce changes characteristic of Alzheimer's disease [29]. Such BBB breakdown contributes to neuronal dysfunction and loss in neurodegenerative disorders [28, 44], common in the aging population of developed countries. Vascular abnormalities are thought to play a role in cerebrovascular diseases [26], one of which is carotid artery stenosis. Bilateral stenosis (> 70% of artery lumen) of carotid arteries requires surgery-carotid endarterectomy (CEA). Neurocognitive disfunctions occur in 25% of patients undergoing CEA due to transient brain ischemia [13].

Bilateral clamping of carotid arteries (BCCA) in rodents, though different from global cerebral ischemia is used as a model of transient incomplete cerebral ischemia [39] and has been shown to produce metabolic and neurotransmitter disturbances in brain areas participating in memory processes and movement control [9]. As humans live longer and remain engaged in a legion of activities, including gardening and pesticide use, an interesting question arises. Can transient incomplete cerebral ischemia exacerbate the toxic effect of exposure to cypermethrin?

The purpose of present study was to extend our knowledge of the action of cypermethrin on memory, movement activity and movement co-ordination in an animal model of transient incomplete cerebral ischemia. The aging human population is chronically exposed to cypermethrin through food consumption (pesticide residues in fruits and vegetables) and use of cypermethrin formulations to control domestic insect pests [8]. There is recent Polish data that cypermethrin residues exceed the national Maximum Residue Level for the substance in 6% of examined fruits [32]. Moreover, cypermethrin residues were detected in drinking water samples collected from dug wells, deep wells and water mains in rural areas of central Poland [3].

Therefore investigation of the possible effect of cypermethrin on memory and movement in mammals exposed to transient brain ischemia is an extremely interesting issue of practical significance.

Materials and Methods

Animals

Non-gravid female Albino-Swiss mice weighing 18–24 g approximately six weeks of age purchased from a licensed dealer (T. Górzkowski, Warszawa, Poland) were used in the study. All the animals were given a seven-day acclimation period and were maintained on a 12-h light/dark photoperiod. Food and tap water were provided *ad libitum*. Temperature was maintained at 21 ± 2°C.

There were four groups of ten animals each: I BCCA-operated and injected 24 h after surgery with 0.1 LD_{50} cypermethrin (BCCA/CY), II BCCA-operated injected 24 h after surgery with respective volume of bi-distilled water (BCCA/H_2O), III sham-operated (with their carotids separated, but not clamped) injected 24 h after surgery with 0.1 LD_{50} of cypermethrin (Sham/CY), and IV sham-operated injected 24 h after surgery with bi-distilled water (BCCA/H_2O). Cypermethrin and bi-distilled water were administered intraperitoneally (*ip*).

Drugs

The surgical procedure of bilateral clamping of carotid arteries (BCCA) was performed under ketamine (100 mg/kg, *ip*) + xylazine (20 mg/kg, *ip*) anesthesia. Ketamine (ketanest, 50 mg/ml in vials á 10 ml) was purchased from Parke-Davis, Berlin, Germany. Xylazine (rometar, 20 mg/ml in vials á 50 ml) was purchased from Spofa, Praha, Czech Republic. Cypermethrin (technical grade 98.2%) as pulvis in vials

á 50 g was purchased from Industrial Organic Chemistry Institute Annopol, Poland.

Tap water was bi-distilled at Hygiene Department of Medical University, Lublin, Poland.

Tween 60 for cypermethrin suspension was purchased from Laboratoriums Reagenzien, Germany.

Surgical procedure

All the experimental procedures were approved by the Local Ethics Committee for Animal Experiments.

Mice were subjected to bilateral clamping of common carotid arteries (BCCA) by wrapping threads around common carotid arteries to occlude blood flow for 30 min. The cessation of carotid blood flow was controlled visually. After 30 min the threads were removed, arteries were inspected for blood re-flow, and the surrounding skin was sutured. Sham-operated mice had their carotids exposed, but not clamped. During the procedure mice were breathing spontaneously and were kept at constant temperature of 37°C by a heating pad and lamp.

Administration of cypermethrin. Sequence of tests

Twenty four hours after surgery, groups I (BCCA/CY) and III (Sham/CY) were injected with 0.1 LD_{50} of cypermethrin *ip*. Groups II (BCCA/H_2O) and IV (BCCA/H_2O) were injected with respective volumes of bi-distilled water *ip*. LD_{50} of cypermethrin was calculated with use of a computer program based on Lichtfield and Wilcoxon's method [18]. LD_{50} of cypermethrin is 169.9 (151.9–190.1) mg/kg, *ip*. 0.1 LD_{50} of cypermethrin was suspended in 10 ml of bi-distilled water with two drops of Tween 60.

Thirty minutes after the injection, animals from all groups (I–IV) were trained in passive avoidance task (PA). Twenty four hours later they were examined in PA for 180, immediately after which they were examined in Y-maze for 8 min, then their locomotor activity was measured within two subsequent 30 min periods and they were then placed on a rotating rod for 120 s to test their movement co-ordination.

Passive avoidance

A step-through PA task was used in the study. The task relies on the innate preference of rodents for dark, enclosed spaces and is regarded as a measure of long-term memory retention [41]. Avoidance training consisted of a single trial in which each animal was placed in an illuminated box (15 × 12 × 15 cm) adjacent to a darkened one (the same size) with an electric grid floor. Thirty seconds after placing the animal in the centre of the illuminated box a passage joining the two boxes was opened. After entering the dark box the animal was exposed to an electric foot shock (2 mA for 2 s). Twenty four hours after the training trial, a memory retention test was conducted in which the same animals were placed in the illuminated box and the latency to enter the darkened box was recorded. The test ended when the mouse entered the darkened box or when 180 s had elapsed.

Y-maze task

Spontaneous alternation was assessed in a Y-maze, which is used as a measure of working spatial memory [21]. The Y-maze consists of three 10 × 10 × 10 cm compartments joined together with 4-cm long corridors at 120° in such a way that each corridor opens to one compartment only. The maze has no floor. It is placed on a clean sheet of paper on a table-top. In order to prevent odor cues, a clean sheet of paper is used for each animal. Mice naturally tend to explore the maze by systematically entering each arm. The ability to alternate requires that the animals know which arm they have already visited. In the task, each mouse was placed at the end of one arm and allowed to move through the maze for eight minutes. The percentage of alternation (defined as consecutive entries into all three arms without repetitions in overlapping triplet sets, to all possible alternations × 100%) was counted. For example, if the arms were marked as X, Y and Z and the animal entered the arms in the following order XYZXZYZXYXYZXZ, the actual alternation would be seven, the total number of arm entries would be fourteen and the percent alternation would be 58.33%.

Locomotor activity

Horizontal spontaneous locomotor activity was assessed with an automated device consisting of a round box (30 cm in diameter) with two photocells mounted horizontally 2 cm above the floor at the angle of 90°. The photo-beam was activated when the mouse interrupted the beam. Animals were not habituated to the apparatus. Therefore, the testing procedure consisted of two consecutive 30 min periods. The first period

was considered as a rate of exploratory locomotor activity. The second period was considered as a rate of spontaneous locomotor activity.

Movement co-ordination

Movement co-ordination was examined on a rotating rod (10 rotations/min). The animals were placed on the rotating rod (1 cm diameter) 50 cm above the ground for 120 s. The trial ended when the mouse fell off the rod or 120 s had elapsed, whichever occurred first.

Statistical analysis

A Kruskal-Wallis non-parametric ANOVA followed by Dunn's multiple comparisons test was used to analyse the data from PA test. PA results were expressed as median values. The results from the Y-maze task, movement co-ordination test and spontaneous motor activity test were shown as the means ± SEM and evaluated by one-way ANOVA followed by Duncan's *post-hoc* test for multiple comparisons. p value < 0.05 was considered statistically significant.

Results

The effect of 0.1 LD_{50} of cypermethrin and BCCA on memory retention tested in passive avoidance task

We observed no statistically significant differences in memory retention among groups I–IV. Median values of latency were 180 s for all the groups.

The effect of 0.1 LD_{50} of cypermethrin and BCCA on working spatial memory in Y-maze task

There were no significant differences in working spatial memory observed among the examined groups. Results obtained were (% of logical alternation behavior expressed as the mean ± SEM): I (BCCA/CY) 61.42 (3.685), II (BCCA/H_2O) 57.17 (4.856), III (Sham/CY) 59.41 (2.853), IV (Sham/H_2O) 64.47 (2.36).

The effect of 0.1 LD_{50} of cypermethrin and BCCA on locomotor activity

Spontaneous movement activity assessed within the first 30 min of observation differed significantly among groups: I (BCCA/CY) *vs.* IV (Sham/ H_2O) ($p < 0.05$), and I (BCCA/CY) *vs.* II (BCCA/ H_2O) ($p < 0.05$). In the subsequent 30 min of observation no statistically significant differences were found among the examined groups and the average locomotor activity in respective groups was lower than within the first 30 min (Fig. 1).

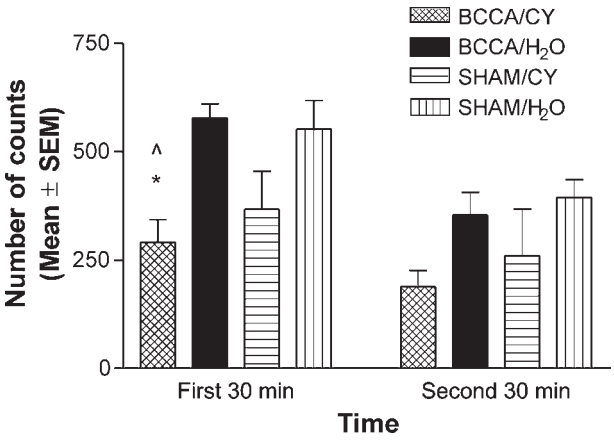

Fig. 1. The influence of 0.1 LD_{50} of cypermethrin *ip* and BCCA on spontaneous movement activity within two subsequent 30 min periods of observation. Columns represent the means ± SEM. Number of mice in each group (n) was 10. * $p < 0.05$ *vs.* Sham/H_2O within the first 30 min of observation, ^ $p < 0.05$ *vs.* BCCA/H_2O within the first 30 min of observation (ANOVA, Duncan's test)

The effect of 0.1 LD_{50} of cypermethrin and BCCA on movement co-ordination

All the animals from groups I–IV demonstrated fully co-ordinated movement on the rotating rod and all remained on the rod for 120 s (means in groups I–IV = 120 s, SEM in groups I–IV = 0).

Altered behavior after *ip* injection of 0.1 LD_{50} of cypermethrin in the examined mice

Immediately after *ip* administration of 0.1 LD_{50} of cypermethrin the animals from group I (BCCA/CY) crowded in one corner of the cage and demonstrated less exploratory activity than before the injection.

Animals from group III (Sham/CY) after cypermethrin injection produced loud sounds, were irritated, jumped high and were hypersensitive to loud sounds. Salivation was observed in a few mice.

Discussion

BCCA is accepted as an animal model of transient incomplete cerebral ischemia [15] and has been shown to cause numerous metabolic and neurotransmitter alterations in rodents [39]. BCCA disturbs the cholinergic system in the rat's brain [11], causes free radical formation, produces changes in glutaminergic system [12] and increases GABA content in the vulnerable structures of rat's brain [35]. BCCA was also found to exacerbate memory impairment caused by exposure to lead [20] and cadmium [19].

N-methyl-D-aspartate (NMDA) receptor antagonists are also known to impair working memory in spatial orientation tasks [5]. BCCA was found to increase the susceptibility to competitive [14] and non-competitive NMDA receptor antagonists [15].

Ketamine, the anesthetic used before the surgical procedure in the present study, is a non-competitive NMDA receptor antagonist [24]. Single *ip* administration of 20 mg/kg of ketamine in rats was found to increase prefrontal acetylcholine (ACh) release by 250% and impair performance in attention tasks [24]. In healthy humans ketamine was found to produce psychotogenic effects including brief and reversible positive and negative symptoms and memory impairment [1]. Ketamine is used in animal models to study "positive" and "negative" symptomatology of schizophrenia [25]. However, ketamine and xylazine anesthetic combination has found multiple uses in veterinary medicine [2, 4, 23, 34, 40]. In the present study, no statistically significant memory impairment was observed. Since ketamine was used for short anesthesia 48 h before memory retention and fresh spatial memory examination, it was assumed that its influence on memory processes was short-lived and insignificant at the time the animals performed memory tasks. Moreover, all the groups of animals tested were exposed to equal doses of ketamine per unit of body weight and memory retention and alternation behavior were not significantly decreased in any groups of mice.

It is known that cypermethrin's toxicity results from its damaging effect on the central nervous system [36]. Even a single application of cypermethin can produce apoptotic cell death in the central nervous systems of exposed animals [6]. The concentration of cypermethin in rabbit brain after a single administration of a subtoxic dose of the pyrethroid was found to be different in brain stem and spinal cord (fast intake and release with maximum concentration at 30 min) compared with cerebral cortex and cerebellum (slower uptake and release with lower maximum concentration) [16].

Results obtained in the current study indicate that neither transient incomplete cerebral ischemia nor single application of 0.1 LD_{50} of cypermetrin *ip* produce significant memory deficits and change in movement co-ordination.

Significant impairment of spontaneous movement activity observed in mice within the first 30-min period of examination after incomplete brain ischemia and exposure to cypermethrin was transient. Jóźwiak et al. assessed locomotor activity in mice subjected to BCCA in a Y-maze but found no significant differences between BCCA- and sham-operated animals [15]. Łukawski et al. also compared locomotor activity of BCCA- and sham-operated mice in a Y-maze [19]. No significant differences were observed among the tested groups. The data obtained in our study are consistent with earlier observations in mice, where ketamine + xylazine anesthesia was used before BCCA procedure, and neither memory processes, nor locomotor activity were found to be decreased by the anesthetics [19, 20].

Little is known about influence of subtoxic doses of cypermethrin on movement co-ordination in mice. However, acute intoxication with type II pyrethroids was found to produce potentiated skeletal muscle contraction in rodents [30], increase extensor tone in the hind limbs as well as cause rolling gait, and movement incoordination progressing eventually to coarse tremors [30, 31]. Choreoform movements of the limbs and tail, typically described in type II acute pyrethroid poisoning in rodents [30, 31], produce movement co-ordination impairment. In the present study, low dose (0.1 LD_{50}) of cypermethrin was used and no such movement co-ordination impairment was seen.

In conclusion, the current study indicates that incomplete brain ischemia combined with exposure to subtoxic dose of cypermethrin transiently impairs spontaneous movement activity in mice. Memory re-

tention, fresh spatial memory and movement coordination were not impaired. These findings are of interest, since elderly humans experiencing transient ischemic attacks are chronically exposed to cypermethrin, used for household pest control or *via* contaminated fruits, vegetables and drinking water.

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