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Effect of transient cerebral oligemia combined with manganese intoxication on memory processes in mice

Manganese (Mn) is well known as an essential trace element due to its role as a cofactor for enzymes such as manganese superoxide dismutase, arginase and pyruvate carboxylase (1). On the other hand, high levels of manganese may produce neurotoxicity. Animals exposed to excessive manganese develop neurological abnormalities, and neuropathological lesions in the brain mainly in the globus pallidus with decreased concentrations of dopamine in the brain (2). Manganese toxicity from industrial exposure can cause a clinical syndrome, "manganism", a Parkinson-like condition related to impaired dopaminergic system by manganese (3). High manganese levels in hair of children with neurodevelopmental deficits suggest that these deficits could be due to Mn-induced neurotoxic effects (1). Further, experiments on animals show that manganese poisoning produces reversible derangement of the learning processes (4). In the current study, we evaluated the influence of manganese on cognitive function in BCCA mice by the use of two tests of learning and memory which were Y-maze spontaneous alternation task and passive avoidance task. In the Y-maze task, spontaneous alternation of mice is regarded as a measure involving spatial working memory (5). The step-through passive avoidance task may give information about acquisition (learning) and recall (retrieval), which are components of long-term memory (6).

It is known that ischemia-induced activation of neurotransmitter systems in the brain is an important process related to the development of ischemic neuronal damage (7). In contrast to cerebral ischemia, cerebral oligemic hypoxia as produced by bilateral clamping of carotid arteries (BCCA) generally does not produce neuronal necrosis in vulnerable brain structures including CA1 field of the hippocampus (8). However, transiently reduced oxygen supply induced by BCCA has been shown to cause metabolic and neurotransmitter alterations of certain brain areas in rodents (9). Cerebral oligemic hypoxia causes disturbances in cholinergic (8), GABAergic (10) and dopaminergic (11) systems in the brain. Moreover, behavioral consequences could be observed in BCCA model like spatial learning deficiencies in a water maze (12). The BCCA model seems to be appropriate for studying the functional consequences of a rather moderate reduction in cerebral blood flow (10) and is thought to reflect the most typical features of ischemic attacks in humans (13). We recently demonstrated that the toxicity of cadmium (14) and lead (15) could be influenced by cerebral oligemia. Since manganese can be neurotoxic it was of significance to investigate cognitive profile in mice exposed to manganese and cerebral oligemic hypoxia.

MATERIAL AND METHODS

Animals and drugs. The study was performed on female Swiss mice, weighing 20–26 g. The animals were kept under standardized laboratory conditions with free access to food and tap water in a room with a 12 h light/dark cycle. The following drug was used: manganese chloride ($MnCl_2 \cdot 4H_2O$) (PPH Polskie Odczynniki Chemiczne, Gliwice) administered intraperitoneally (i.p.). Manganese chloride was injected at a dose of 15.9 mg/kg that was 10% of two-week mean lethal dose (LD_{50}). The drug was administered in a volume of 10 ml/kg. All experimental procedures run in this study were approved by the University Ethics Committee for Animal Experiments.

Surgical procedure. Mice were subjected to 30 min of bilateral clamping of the common carotid arteries (BCCA) by using thread. The BCCA procedure was carried out under ketamine (Ketanest, Parke-Davis, Berlin, 50 mg/ml, i.p.) + xylazinum (Rometar, Spofa, Praha, 20 mg/ml, i.p.) anesthesia. The cessation of carotid blood flow was controlled visually. After the occlusion period the threads were removed and the surrounding skin was sutured. Sham-operated animals had their carotid arteries exposed for the same period of time without clamping. During anesthesia and surgery, the mice were breathing spontaneously and the rectal temperature was kept at 37°C by a heating pad. Each task was performed on the following groups: sham, BCCA, sham + Mn and BCCA + Mn.

Passive avoidance performance. The step-through passive avoidance task was used in the current study. The passive avoidance test is generally regarded as a measure involving long-term memory (6). Mice were trained in the passive avoidance task 24 h after BCCA or sham surgery and manganese was injected once at a dose of 15.9 mg/kg 60 min before training or retention test. Avoidance training consisted of a single trial in which the mice were individually placed in an illuminated box (15x12x15 cm) connected to a darkened box (15x12x15 cm) that was equipped with an electric grid floor. A 4x5 cm doorway was located at the floor level in the center of the common wall. Immediately, after the mouse entered the darkened box, it was punished by an electric foot shock (0.6 mA for 2 s). Twenty-four hours after the training trial (on the 2nd post-surgical day) the retention test was conducted in which the same animals were put into the illuminated box and the latency to enter the darkened box was recorded. The trial ended when the mouse entered the darkened box or until 180 s had elapsed, whichever occurred first. Mice that did not enter in the time allotted received a latency score of 180 s.

Y-maze task. Spontaneous alternation was assessed in the Y-maze task (5). The Y-maze was made of three compartments measuring 10x10x10 cm, which did not have a floor and it was placed on a clean sheet of paper. A clean sheet of paper was used after each animal to prevent odor cues. Each mouse was placed at the end of one arm and allowed to move freely through the maze during an 8-min session. Alternation (defined as consecutive entries into all three arms without repetitions) and the total number of arm entries (locomotor activity) were scored. Locomotor activity was collected cumulatively over 8 min. The percent alternation was calculated as the ratio of actual to possible alternations (defined as the total number of arm entries – 2) x 100. Alternation behavior was examined after BCCA or sham surgery on the 2nd post-surgical day. Manganese was given once at a dose of 15.9 mg/kg, 60 min before the test session.

Statistics. A Kruskal-Wallis non-parametric ANOVA followed by Dunn's multiple comparisons test was used to calculate results from the passive avoidance task and the Y-maze. Group differences were considered statistically significant at $p < 0.05$.

RESULTS

The latency in the passive avoidance task and Y-maze performance were not affected in mice subjected only to BCCA (Tables 1, 2 and 3). The combination of manganese (15.9 mg/kg) with BCCA did not impair learning (Table 1) and recall (Table 2) in the passive avoidance task although a tendency towards decreased latency (statistically not significant) was present when manganese was given before the retention test (Table 2). Similarly, BCCA combined with manganese (15.9 mg/kg) did not influence spontaneous alternation (Table 3) and locomotion (data not shown) in the Y-maze.

Table 1. The effect of manganese on learning in the passive avoidance task

	Sham n = 10	BCCA n = 9	Sham + Mn (15.9 mg/kg) n = 10	BCCA + Mn (15.9 mg/kg) n = 10
Latency (s)	180 (180, 180)	145 (67, 180)	165 (46, 180)	180 (45, 180)

Data are presented as median values with the 25th and 75th percentiles. n – number of mice. Kruskal-Wallis test followed by Dunn's test

Table 2. The effect of manganese on recall in the passive avoidance task

	Sham n = 11	BCCA n = 10	Sham + Mn (15.9 mg/kg) n = 9	BCCA + Mn (15.9 mg/kg) n = 10
Latency (s)	180 (150, 180)	176 (116, 180)	123 (46, 180)	69 (36, 180)

Data are presented as median values with the 25th and 75th percentiles. n – number of mice. Kruskal-Wallis test followed by Dunn's test

Table 3. The effect of manganese on spontaneous alternation in the Y-maze

	Sham n = 10	BCCA n = 9	Sham + Mn (15.9 mg/kg) n = 10	BCCA + Mn (15.9 mg/kg) n = 10
Alternation (%)	60.2 (56, 67)	51.7 (48, 63)	58.5 (52, 64)	53.0 (47, 59)

Data are expressed as median values with the 25th and 75th percentiles. n – number of mice. Kruskal-Wallis test followed by Dunn's test

DISCUSSION

It has been demonstrated that cerebral oligemic hypoxia induced by BCCA reduces blood flow to oligemic levels without producing neuronal necrosis in brain vulnerable structures (8). However, BCCA alone can cause memory deficits in spatial memory tasks like a water maze (9). Additionally, BCCA in combination with iron treatment led to pronounced deficits of spatial memory in this test (16). We reported that co-exposure to cerebral oligemia and cadmium or lead can cause impairment in passive avoidance and alternation behavior (14, 15). In the present study, acute treatment with manganese did not impair memory function in BCCA mice, although a tendency (statistically not significant) towards decreased retention in the passive avoidance task in the BCCA + Mn group was visible (Table 2). Therefore, further studies are needed to evaluate the influence of chronic manganese treatment on learning and memory in mice subjected to cerebral oligemia. It has been reported that chronic exposure to manganese can disturb cognitive function both in rodents (4) and non-human primates (17). As presented in the tables, acute exposure of manganese does not seem to be amnesic

in the passive avoidance task and Y-maze test even in BCCA mice which are known to be more vulnerable to environmental toxins (14, 15).

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SUMMARY

Manganese is an essential trace element due to its role as a cofactor for a number of enzymes. On the other hand, some individuals exposed to high levels of manganese in their work can develop neurotoxic symptoms. The aim of the present study was to examine the effect of manganese on memory processes in mice exposed to transient cerebral oligemic hypoxia. Cerebral oligemia was induced by bilateral clamping of carotid arteries (BCCA) for 30 min under anesthesia. Long-term memory was evaluated using the passive avoidance task while spontaneous alternation was assessed by means of the Y-maze task. In the passive avoidance task, treatment with manganese (15.9 mg/kg i.p.) did not impair retention in BCCA mice. Manganese at the same dose of 15.9 mg/kg i.p. did not alter spontaneous alternation in the Y-maze either. These results show that cerebral oligemic hypoxia does not affect memory function of mice exposed to acute manganese intoxication.

Wpływ łącznego działania przejściowej oligemii mózgu oraz intoksykacji manganem na procesy pamięci u myszy

Mangan jest mikroelementem niezbędnym w organizmie człowieka z powodu roli kofaktora, jaką pełni dla wielu enzymów. Z drugiej strony u niektórych osobników narażonych na wysokie stężenia manganu w środowisku pracy mogą wystąpić objawy neurotoksyczności. Celem pracy było zbadanie wpływu manganu na procesy pamięci u myszy poddanych przejściowej oligemicznej hipoksji mózgu. Oligemia mózgu była wywołana metodą obustronnego zamknięcia tętnic szyjnych wspólnych (BCCA) na okres 30 min. w znieczuleniu ogólnym. Pamięć długotrwała była badana w teście biernego unikania, natomiast spontaniczną alternację oceniano za pomocą labiryntu Y. W teście biernego unikania podanie manganu (15,9 mg/kg i.p.) nie zaburzyło retencji u myszy BCCA. Mangan w tej samej dawce 15.9 mg/kg i.p. nie wpłynął także na spontaniczną alternację w labiryncie Y. Wyniki te dowodzą, że oligemiczna hipoksja mózgu nie wpływa na pamięć u myszy poddanych ostrej intoksykacji manganem.