Polish Journal of Environmental Studies Vol. 9, No. 4 (2000), 301-304

Neurotoxic Effect of Copper Salts in Rats

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Received: November 15, 1999
Accepted: December 14, 1999

Abstract

Neurotoxic effects of copper ions after intraperitoneal (ip) and intracerebroventricular (icv) injections of $CuSO_4 \cdot 5H_2O$ and $Cu(CH,COO)_2 \cdot H_2O$ at doses of 1-100 nmols icv and 0.1-100 μmols/kg ip was determined in rats using two behavioural methods: exploratory and locomotor activity in an open field test and spatial memory in a water maze test. It has been found that higher doses of copper salts (100 nmols icv and 100 μmols/kg ip) significantly decreased spatial memory of rats.

Keywords: copper ions, neurotoxic effects in rats, spatial memory, water maze test.

Introduction

There are many data on the neurotoxic effects of a number of metals and organometallic compounds [1]. Deposition of excess copper in basal ganglia exerts neurotoxic effects manifested as signs of Wilson's disease. It was also proposed to use copper-loaded rats as a model of Wilson's disease [2]. On the other hand, a perinatal copper deficiency in rats leads to severe reduction of copper levels in liver and in all brain regions except to hypothalamus [3], and contemporary reduction of norepinephrine and dopamine concentrations in the striatum and other brain areas [3]. It has been previously found in our laboratory that copper complex LHRH (as well as copper acetate introduced into the lateral brain ventricle) displayed convulsive effects in rats [4].

The aim of the present studies was to determine the neurotoxic effect of copper ions introduced (as sulfuric and acetate salts) in single or repeated icv and ip doses by means of open field and water maze tests. We especially focused on their influence on spatial memory using the water maze test.

Material and Methods

Animals

The study was performed on Wistar albino rats of both sexes weighing 250-300 g kept on 12:12 L:D cycle (light from 6 am to 6 pm) with free access to water and standard food (Murigran Motycz-Lublin, Poland) obtained from the Animal Farm of the Medical University of Silesia. All studies were performed during the light portion of the L:D cycle between 9 am and 2 pm. The animals were allowed two weeks of adaptation to the laboratory conditions before experiments.

Intracerebroventricular (icv) Cannulation

One week before experiments the polyethylene cannulas (length 25 mm, external diameter of 0.6 mm) (Tomel, Tomaszow Maz., Poland) were implanted under thiopental anaesthesia (40 mg/kg ip) into the right lateral brain ventricle (icv) using the following coordinates: depth 4 mm from the surface of the skull, 2 mm to the right from sagittal suture and 2 mm caudally from coronary suture. The cannulas were fixed to the skull bones with acrylic glue Deltamed PM 16 (Chemical Factory, Oswiecim, Poland).

The animals receiving intraperitoneal (ip) injections of copper salts did not require any additional arrangements for experiments.

Water Maze Aparatus

The maze is constructed of plexiglass in a form of a box 80 cm long, 57 cm wide and 38 cm high. At each angle of the box there is a compartment of the following dimensions: 25 cm long and 18 cm wide with 7.5 cm gate.

Only one compartment is equipped with an escape platform placed 0.5 cm above the water surface. The entire maze is filled with water 27°C, 18 cm deep (Fig. 1).

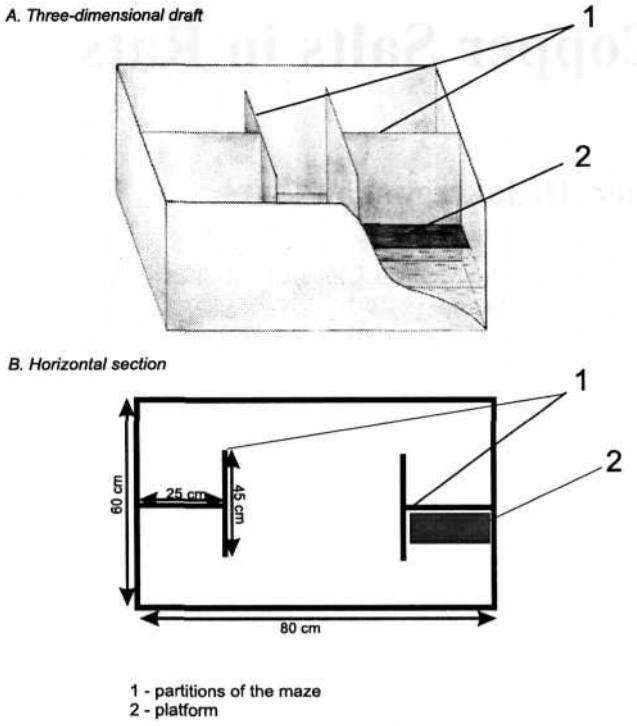

Fig. 1. Diagram of the water maze for evaluating spatial memory in rats.

Open Field Test

A standard open field according to Janssen et al. [5] was used to determine rats' exploratory and locomotor activity.

Procedure

24 h before the experiment rats were trained to record a position of a compartment equipped with an escape platform. A single animal was placed in a central part of the maze and swam looking for the platform. When the animal reached the escape platform it was allowed to stay there for 1 min. and was then subjected to the next trial. The training session consisted of three trials. Non performer animals which were unable to find the platform or needed as long as 5 min. to reach the platform were eliminated from the experiment.

Experimental Session

On the day of the experiment animals were icv or ip injected with copper sulfate ($CuSO_4$-$5H_2O$; POCH Gliwice, Poland) or copper acetate ($Cu(CH_3COO)_2 \cdot H_2O$)* at doses of icv: 1 to 100 nmols and ip: 0.1 to 100 µmols/kg, and 30 or 20 min. after injections of these salts animals were subjected to three succesive probe trials performed in the same way as the day before in a trial session. At every probe the latency time between placing the animal into a central part of the maze and its climbing onto the platform was measured. A mean latency time for three probe trials was calculated.

An additional group of rats was used to study the effect of copper salts icv injections on rat behaviour using the open field test.

At the end of the experiment rats were sacrificed by chloralhydrate overdosing (500 mg/kg ip) and the proper localization of implanted cannulas was checked by injections of 1% methylene blue solution and the visual inspection in the lateral brain ventricle was performed.

Statistics

The results were analyzed using Dunnett's test following significant ANOVA.

Results

lev injections of $CuSO_4$ at doses of 1 and 10 µmols did not induce any significant effect on spatial memory in a water maze test (Fig. 2) as well as on rats' behaviour determined in an open field test (Fig. 5). After icv injection of single dose of 100 nmols of $CuSO_4$ a transient, lasting 5-15 min. convulsions were observed in about half the animals of the group. The same dose (100 nmols) single as well as repeated once daily for 3 days significantly inhibited rats' performance in the water maze test (Figs. 3, 4).

The same inhibitory effect on rat's memory determined by means of a water maze test was also observed after icv injections of copper acetate in a dose of 100 nmols while lower doses of 1 and 10 nmols were without any significant effect (Fig. 8).

Copper sulfate injected intraperitoneally (ip) at doses of: 0.1, 1, and 10 µmols/kg had no significant effect on rat's spatial memory in a water maze test (Fig. 6), but the same salt injected ip in a dose of 100 µmols/kg significantly increased latency time in this test (Fig. 6).

The dose of 10 µmols/kg ip of $CuSO_4$ repeated daily in three succesive days also significantly prolonged latency time of animals in this test (Fig. 7).

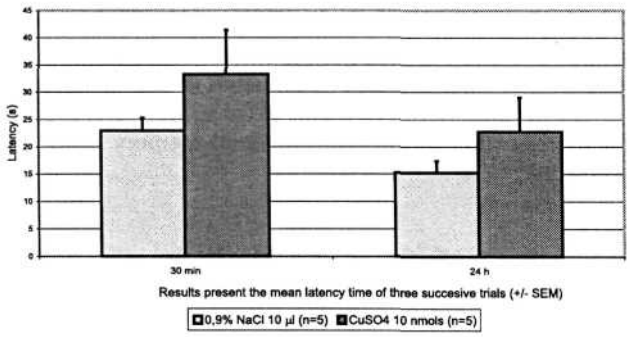

Fig. 2. Efect of intracerebroventricular (icv) injection of copper sulfate at a dose of 10 nmols on spatial memory of female rats determined in a water maze test.

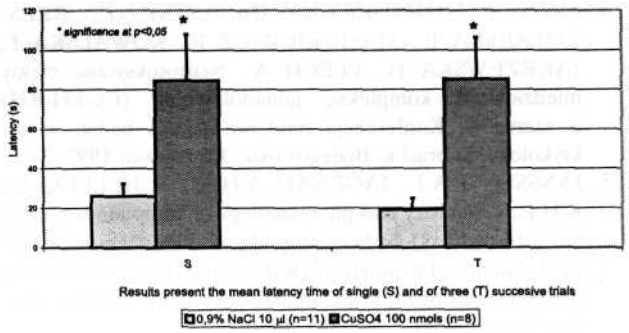

Fig. 3. Effect of icv injection of copper sulfate at a dose of 100 nmols on spatial memory of female rats determined in a water maze test.

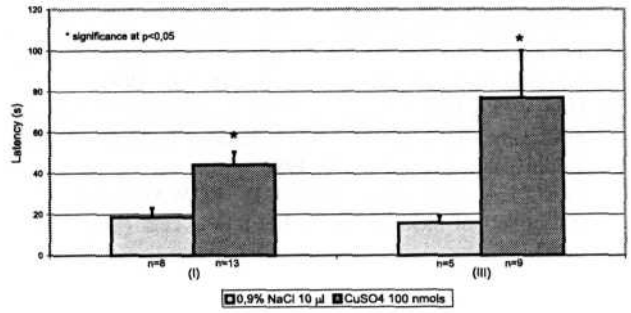

Fig. 4. Effect of the single (I) and three, daily repeated (III) icv doses of 100 nmols copper sulfate on spatioal memory of male rats determined in a water maze test.

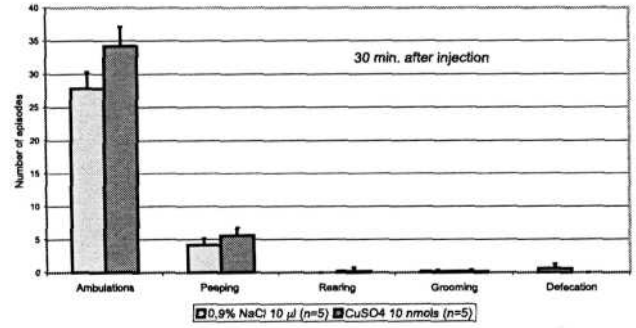

Fig. 5. Effect of intracerebroventricular (icv) injection of copper sulfate at a dose of 10 nmols on rat's behaviour measured with an open field test.

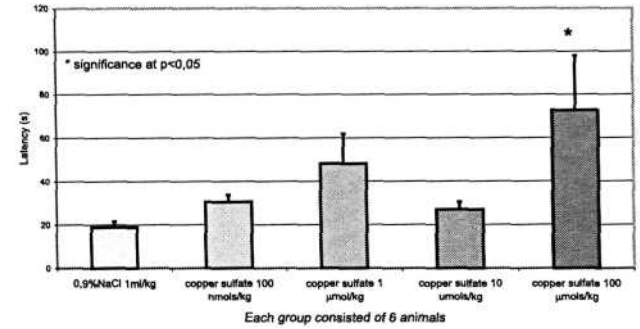

Fig. 6. Effect of copper sulfate on spatial memory determind in a water maze test 20 min. after ip injection of each dose. Results present the mean latency time of three successive trials of female Wistar rats.

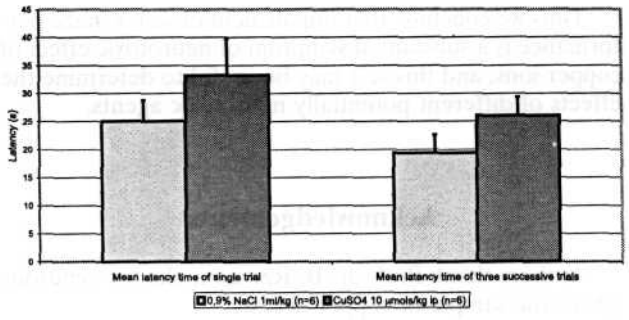

Fig. 7. Effect of intraperitoneal (ip) injection of copper sulfate on spatial memory determined by means of a water maze test. Results present the mean latency time of the single and of three successive trials of male Wistar rats.

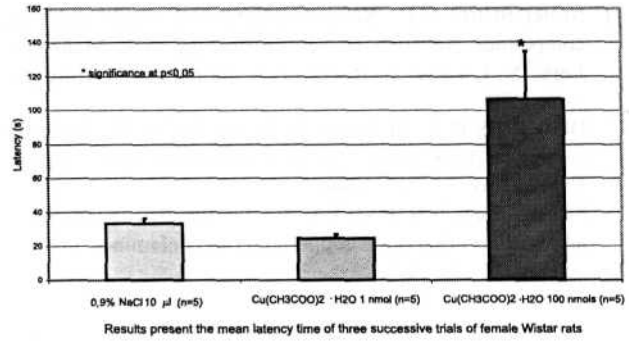

Fig. 8. Effect of copper aceate on spatial memory determind in a water maze test 30 min. after intracerebroventricular (icv) injection.

Discussion

The results presented here indicate that higher doses of copper salts either sulfate or acetate impaired spatial memory of rats. This effect is due to copper ions as it was induced by two different copper salts: sulfate and acetate in two ways of administration e.g.: directly into the lateral brain ventricle and intraperitoneally. The stronger inhibitory effect of repeated copper doses also observed confirms the role of copper accumulation in depression of spatial memory in rats.

This effect indicates the neurotoxicity of effective doses of copper ions. It may be at least connected with their effect on catecholamines metabolism in rat's brain. It was found out by Saito et al. [7] that NE depression and excessive accumulation of DA occurs in cerebral cortex of Long-Evans Cinnamon rats. Rats of this strain are used as an animal model of Wilson's disease as they appear to have excessive copper accumulation in the brain [8].

Among many tasks for testing spatial memory we propose the use of the water maze, which is more simplified to compare with classical Morris test [6], as it is not necessary to record the way of swimming of an animal in the maze to find the aim platform. In our test determination of the latency time for entering the aim platform placed in one compartment of the maze seems to be a suitable index of trial performance.

It has been recently found in our laboratory, that neurotoxic doses of other metal salts also inhibited rat's spatial memory determined by means of our water maze test [9].

Thus we conclude that impairment of water maze performance is a substantial symptom of neurotoxic effect of copper ions, and this test may be useful to determine the effects of different potentially neurotoxic agents.

Acknowledgements

Authors thank Prof. dr. B. Rzeszotarska for generous gift of the sample of copper acetate.

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