Pharmacology, Biochemistry and Behavior 91 (2009) 430–435

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Diphenyl ditelluride impairs short-term memory and alters neurochemical parameters in young rats

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ARTICLE INFO

Article history:
Received 15 January 2008
Received in revised form 7 August 2008
Accepted 21 August 2008
Available online 28 August 2008

Keywords:
Organotellurium
Na^+/K^+ATPase
Object recognition memory
Glutamate uptake and release

ABSTRACT

The aim of this study was to investigate if maternal exposure to 0.03 mg/kg of diphenyl ditelluride $(PhTe)_2$ during the first 14 days of lactational period in Wistar rats alters recognition memory and neurochemical parameters in young rats. Object recognition memory task, evaluation of synaptosomal [^{3}H]glutamate uptake and release as well as cerebral Na^+/K^+ATPase activity were evaluated in 4 week-old rats. There were no significant specific overt signs of maternal intoxication. The body weight gain of rats was similar among groups. $(PhTe)_2$-exposed group showed a significantly lower time exploring the novel object when compared to the performance of the control group in short-term memory (STM) test. In addition, $(PhTe)_2$ significantly inhibited synaptosomal [^{3}H]glutamate uptake and cerebral Na^+/K^+ATPase activity in animals. The synaptosomal [^{3}H]glutamate release was similar between $(PhTe)_2$ and control groups. In conclusion, the present study establishes that young rats presented cognitive impairment after exposure to $(PhTe)_2$ via maternal milk, demonstrated by the performance of animals in object recognition memory task. The possible mechanism involved in $(PhTe)_2$ action in memory of recognition might involve inhibition of cerebral Na^+/K^+ATPase activity and synaptosomal [^{3}H]glutamate uptake.

1. Introduction

Although the tellurium (Te) element rarely occurs in the free state in nature, silver and bismuth tellurides do occur (Larner, 1995a; Schroeder et al., 1967). Moreover, this metallic element is known to be present in plant material, particularly in members of the Alium family, such as garlic (Larner, 1995b). Currently, inorganic Te is used in metal-oxidizing solutions to blacken or tarnish metals (Yarema and Curry, 2005) and in the industry of nanoparticulate semiconductors (Green et al., 2007; Zhang and Swihart, 2007). Moreover, the use of organic Te compounds will increase due to its importance in organic synthesis (Comasseto et al., 1997).

A number of studies have shown that trace amounts of Te are present in body fluids, such as blood and urine (Siddik and Newman, 1988; Newman et al., 1989). Furthermore, Te has been shown to be present as tellurocysteine and telluromethionine in several proteins in bacteria (Boles et al., 1995; Budisa et al., 1995), yeast (Yu et al., 1993) and fungi (Ramadan et al., 1989). But to date, no telluroproteins have been identified in animal cells. By contrast, attention has been drawn to the toxicity of Te.

Nowadays, two cases of toxicity in young children from ingestion of metal-oxidizing solutions that contained substantial concentrations of Te were reported in the literature (Yarema and Curry, 2005). Clinical features of acute Te toxicity include a metallic taste, nausea, blackened oral mucosa and skin and garlic odor of the breath (Muller et al., 1989).

Exposure of experimental animals to Te can cause a variety of toxic effects, including reversible hind limb paralysis due to demyelination of the sciatic nerve and spinal roots (Lampert et al., 1970; Lampert and Garrett, 1971). This has been proposed to be primarily due to blockage of cholesterol biosynthesis at squalene epoxidase (Wagner-Recio et al., 1994), which sequentially affects the transcription of the myelin proteins themselves at the gene level (Morell et al., 1994). Moreover, dietary exposure to high levels (3300 ppm) of metallic Te causes persistent neuromotor impairment which is associated with a severe deficit in shock avoidance. Furthermore, Te could also cause a lowered sensitivity to noxious stimulus, which in turn would retard the learning of the active avoidance task (Dru et al., 1972). Sodium tellurite intoxication causes a consistent deficit in a non-aversive spatial learning in water maze task that could not be overtly linked to motor or motivational impairment in Te exposed animals (Widy-Tysziewicz et al., 2002). Dimethyltellurium, an important compound derived from inorganic Te metabolism in mammals, has been reported as an inducer of peripheral neuropathy in rats (Goodrum, 1998). Moreover, data from our research group suggest that exposure of mothers to low doses of diphenyl ditelluride $(PhTe)_2$, an organotellurium compound, may result in disinhibitional behavior of their offspring on elevated plus maze task (Stangherlin et al., 2006). Besides, $(PhTe)_2$ can be teratogenic, causing various morphologic abnormalities in rat fetuses in development (Stangherlin et al., 2005).

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doi:10.1016/j.pbb.2008.08.020

Table 1
Behavioral and neurochemical experimental protocol

	Postnatal days				
	0–21		21–31		
	0–14	14–21	21–27	28–30	31
Dams	0.03 mg/kg/day $(PhTe)_2$ or canola oil	–	–	–	–
Litter	–	–	–	Behavioral analysis	Neurochemical analysis
	Suckling		Weaned		

Of particular importance, our research group has obtained persuasive evidences indicating that $(PhTe)_2$ causes marked neurotoxic effects in mice after acute or prolonged exposure either by subcutaneous or intraperitoneal routes (Nogueira et al., 2004). $(PhTe)_2$ affects a number of neuronal processes and modifies the functionality of the glutamatergic system in vitro and in vivo (Nogueira et al., 2001) as well as inhibits the cerebral Na^+/K^+ATPase activity (Borges et al., 2005).

Glutamate is known to play an important role in cognition, learning and memory (Davis et al., 1994; Maren, 1996; LeDoux, 1994) and in the neural plasticity of synaptic connections (Kaczmarek et al., 1997). Moreover, Na^+/K^+ATPase is an enzyme embedded in the cell membrane, responsible for the generation of the membrane potential through the active transport of sodium and potassium ions in the central nervous system necessary to maintain neuronal excitability (Erecinska and Silver, 1994).

With regard to behavior, rodents naturally tend to approach and explore novel objects, which are assumed to have no natural significance to the animal and which have never been paired with a reinforcing stimulus (Dere et al., 2007). They also show an innate preference for novel over familiar objects. Rodents readily approach objects and investigate them physically by touching and sniffing the objects, rearing upon and trying to manipulate them with their forepaws (Aggleton, 1985). This behavior can be easily quantified and utilized to study simple recognition memory as well as more complex spatial-, temporal- and episodic-like memory in rodents. The standard object recognition task measures the spontaneous behavior (Dere et al., 2007). The novelty-preference paradigm does not require lengthy training and does not induce high levels of arousal and stress (Ennaceur and Delacour, 1988).

Thus, the present investigation was carried out to determine the effects of $(PhTe)_2$ on the behavioral performance of young rats in object recognition memory task. The possible involvement of glutamatergic system and of cerebral Na^+/K^+ATPase activity in $(PhTe)_2$ effect was also evaluated.

2. Materials and methods

2.1. Materials

Diphenyl ditelluride $(PhTe)_2$ was synthesized according to the literature method (Petragnani, 1994). Analysis of the ^1H NMR and ^{13}C NMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of $(PhTe)_2$ (99.9%) was determined by GC/HPLC. $(PhTe)_2$ is a solid compound, very stable and can be stored in the laboratory, in simple flasks for a long time. $(PhTe)_2$ was diluted in canola oil which was obtained from a standard commercial supplier.

2.2. Animals

Virgin female Wistar rats (180–240 g) from our own breeding colony were used. The animals were kept on a 12 h light/dark cycle, at a room temperature of 22 °C, with free access to food and water. The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, Federal University of Santa Maria, Brazil.

2.3. Experimental procedure

Experimental protocol of exposure was performed as described by Stangherlin et al. (2006). Briefly, sexually naive female rats were mated with male previously tested as fertile (three females and one male in each cage). The onset of pregnancy was confirmed by the presence of sperm in vaginal smears (day 0 of pregnancy) and pregnant dams were immediately housed in individual cages. At birth, the dams received $(PhTe)_2$ (0.03 mg/kg, experimental group) or canola oil (1 ml/kg, control group) via subcutaneous (s.c.) injection once daily during the first 14 days of lactational period. The dose of $(PhTe)_2$ used in this study was selected on the basis of LD_{50} study carried out in our laboratory (Meotti et al., 2003). The body weight of dams and their offspring were recorded during the experimental period. At birth, all litters were culled to eight pups. Whenever possible, only male rats were kept within the litter and females were kept just to maintain equal litter sizes. On 21st postnatal day (PND 21), pups were weaned and placed on *ad libitum* standard rat chow diets. After the 1-week post-weaning period, the object recognition task was conducted (in the morning of PND 28). The observer was blind regarding the group, and the behavioral task was carried out under low-intensity light. Only male rats were used in the behavioral test, litter was invariably constituted of four animals (n=6–8 litters (4 animals each litter)). Twenty-four hours after the last behavioral test, neurochemical analyses were performed (Table 1).

2.4. Behavioral analysis

The object recognition task was performed according to Rosa et al. (2003) with some modifications. The behavioral task was performed in a 45×45 cm open field surrounded by 30 cm height walls, made of brown plywood. All animals were given a habituation session where they were left to freely explore the open field for 5 min. No object was placed in the box during the habituation trial (Fig. 1a). Subsequently, four objects were used: A_1, A_2, B and C. The "A" objects were two identical triangles, the "B" object was a ball and the "C" object was a rectangle. All objects were made of plastic material, with 10 cm×10 cm (length×height). Each object had the pattern of color, as follows: blue, red and yellow. Twenty-four hours after habituation, training was conducted by placing each individual rat for 5 min into the field, in which two identical objects (objects A_1 and A_2) were positioned in two adjacent corners, 10 cm from the walls (Fig. 1b). In a short-term memory (STM) test given 1.5 h after training, the rats explored the open field for 5 min in the presence of one familiar (A)

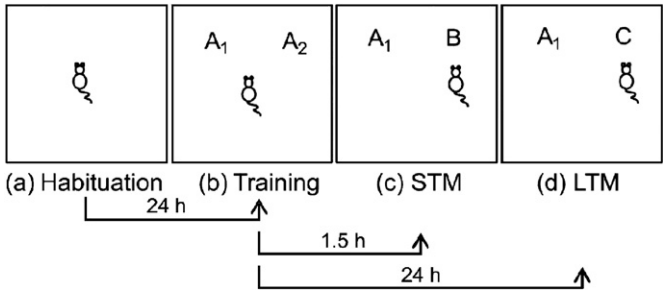

Fig. 1. Behavioral analysis. The object recognition task took place in an open field made of brown plywood. All animals were given to freely explore the open field for 5 min for the *habituation* trial (a); *training* (b) carried out 24 h after habituation; the *short-term memory* (*STM*) test (c) carried out 1.5 h after training; and the *long-term memory* (*LTM*) test (d) carried out 24 h after training. A, B and C represent the objects. Exploratory preference in: Training=$(A_2/(A_1+A_2))\times 100$; STM=$(B/(A_1+B))\times 100$; LTM=$(C/(A_1+C))\times 100$.