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

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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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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)

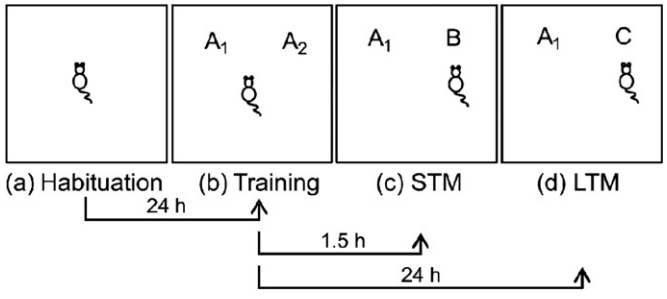

Fig. 1. Behavioral analysis. The object recognition task took place in an open field made of brown plywood. All animals were given to freely explore the open field for 5 min for the *habituation* trial (a); *training* (b) carried out 24 h after habituation; the *short-term memory* (*STM*) test (c) carried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

Table 2
Evaluation of exposure to diphenyl ditelluride

Groups	Control	Diphenyl ditelluride
Body weight (g)—PND28		
Dams	241±15	235±17
Young rats	71±7	67±5[a]
Tremor, garlic odor or loss of hair		
Dams	No signs	
Young rats	No signs	

[a] Exposure to diphenyl ditelluride via maternal milk.

and one novel (B) object (Fig. 1c). All objects presented similar textures, colors, and sizes, but distinctive shapes. The percentage of the total exploration time that the animal spent investigating the novel object was the measure of recognition memory. Between trials the objects were washed with 10% ethanol solution. In a long-term memory (LTM) test given 24 h after training, the same rat explored the field for 5 min in the presence of a familiar object A and a novel object C (Fig. 1d). Recognition memory was evaluated as for the STM test. Exploration was defined as sniffing or touching the object with the nose and/or forepaws. Data are expressed as the mean±SE percentage time exploring any of the objects (training) or the novel objects. Exploratory preference in: Training=$(A_2/(A_1+A_2))\times 100$; STM=$(B/(A_1+B))\times 100$; LTM=$(C/(A_1+C))\times 100$.

2.5. Preparation of synaptosomes

Twenty-four hours after the last behavioral test, three animals of each group were decapitated. After that, the whole brain was removed and used to prepare synaptosomes on a discontinuous Percoll gradient according to Dunkley et al. (1988). Protein concentration was measured according to the method of Lowry et al. (1951).

2.5.1. [^{3}H]glutamate release by synaptosomes

Determination of [^{3}H]glutamate release was accomplished according to the method described by Migues et al. (1999). The synaptosomal preparation was loaded with 0.25 μCi [^{3}H]glutamate (Amersham, specific activity 53 mCi/mmol, final concentration 5 μM) by pre incubation in Tris/HCl buffered salt solution (composition in mM: Tris/HCl 27, NaCl 133, KCl 2.4, $MgSO_4$ 1.2, KH_2PO_4 1.2, Glucose 12, $CaCl_2$ 1.0) pH 7.4 (adjusted with HCl), for 15 min at 37 °C. Aliquots of labeled synaptosomes (1.4 mg protein) were centrifuged at 16,000 ×*g* for 1 min. Supernatants were discarded, and the pellets were washed four times in Tris/HCl buffer by centrifugation at 16,000 ×*g* for 1 min (at 4 °C). To assess the basal release of [^{3}H]glutamate, the final pellet was resuspended in Tris/HCl buffer and incubated for 60 s, at 37 °C. Incubation was terminated by immediate centrifugation (16,000 ×*g*, 1 min, 4 °C). Radioactivity present in supernatants and pellet was separately determined in a scintillation counter. The released [^{3}H]glutamate was calculated as a percentage of the total amount of radioactivity present in the synaptosomes at the start of the incubation period.

2.5.2. [^{3}H]glutamate uptake by synaptosomes

Determination of [^{3}H]glutamate uptake was accomplished according to the method described by Nogueira et al. (2002). The synaptosomal preparation was washed twice by suspending in 3 volumes of 0.3 M sucrose, in 15 mM Tris/acetate buffer (pH 7.4) and centrifuged at 35,000 ×*g* for 15 min. The final pellet was suspended in 0.3 M sucrose, 15 mM Tris/acetate buffer (pH 7.4), and incubated in Tris/HCl buffer (composition in mM: Tris/HCl 27, NaCl 133, KCl 2.4, $MgSO_4$ 1.2, KH_2PO_4 1.2, Glucose 12, $CaCl_2$ 1.0) pH 7.4 (adjusted with HCl), in the presence of [^{3}H]glutamate (final concentration 100 μM) for 1 min at 37 °C. The reaction was stopped by centrifugation (16,000 ×*g*, 1 min, 4 °C), and the pellets were washed three times in Tris/HCl buffer by centrifugation at 16,000 ×*g* for 1 min (at 4 °C). Radioactivity present in pellet was measured in a scintillation counter. Specific [^{3}H]glutamate uptake was calculated as the difference between the uptake obtained in the incubation medium described above, and the uptake obtained with a similar incubation medium in which NaCl was replaced by choline chloride.

2.6. Na^+/K^+ATPase activity

Twenty-four hours after the last behavioral test, 6–8 animals of each group were euthanized, the whole brain was removed and the homogenate was prepared in 0.05 M Tris/HCl buffer (pH 7.4). The homogenate was centrifuged at 4000 ×*g* at 4 °C for 10 min and supernatant was used for assay of protein Na^+/K^+ ATPase. The reaction mixture for Na^+/K^+ATPase activity assay contained 3 mM MgCl, 125 mM NaCl, 20 mM KCl and 50 mM Tris/HCl, pH 7.4, in a final volume of 500 μl. The reaction was initiated by addition of ATP to a final concentration of 3.0 mM. Controls were carried out under the same conditions with the addition of 0.1 mM ouabain. Na^+/K^+ATPase activity was calculated by the difference between the two assays. Released inorganic phosphate (Pi) was measured by the method of Fiske and Subbarow (1925). All the experiments were conducted at least four times and similar results were obtained.

2.7. Statistical analysis

The litter was considered the experimental unit in statistical behavioral analyses performed. For the behavioral analyses, the statistical significance was assessed by analysis of variance (ANOVA) with repeated measures. Post hoc Duncan's test was carried out when appropriated. A value of $p<0.05$ was considered to be significant. For the neurochemical analyses, the statistical significance was assessed by analysis of variance (ANOVA) and post hoc Duncan's test was carried out when appropriated. A value of $p<0.05$ was considered to be significant.

3. Results

3.1. General analysis

There were no significant specific overt signs of maternal intoxication, such as reduction of body weight, tremor, garlic odor and loss of hair. The young rats demonstrated normal body weight gain (Table 2).

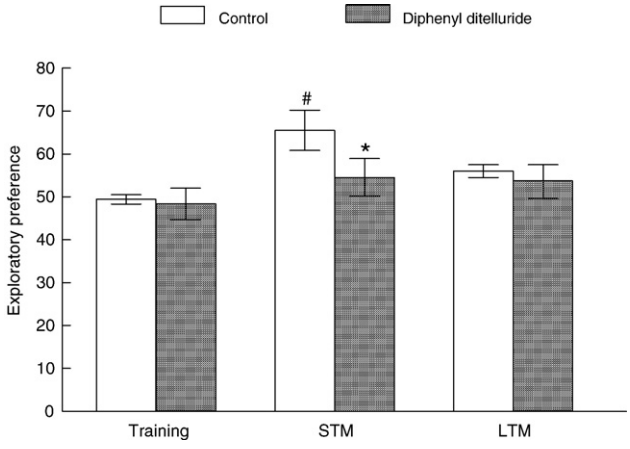

Fig. 2. Evaluation of exploratory preference on object recognition task in young rats during *Training* (percentage of time spent exploring any of the two identical objects), *STM* (percentage of time exploring spent the novel object, test carried out 1.5 h after training) and *LTM* (percentage of time spent exploring the novel object, test carried out 24 h after training). Results are expressed as mean±S.E.M. $n=6$–8 litters (4 animals each litter). #$p<0.05$ compared to the control group during training; *$p<0.05$ compared to the control group during STM.

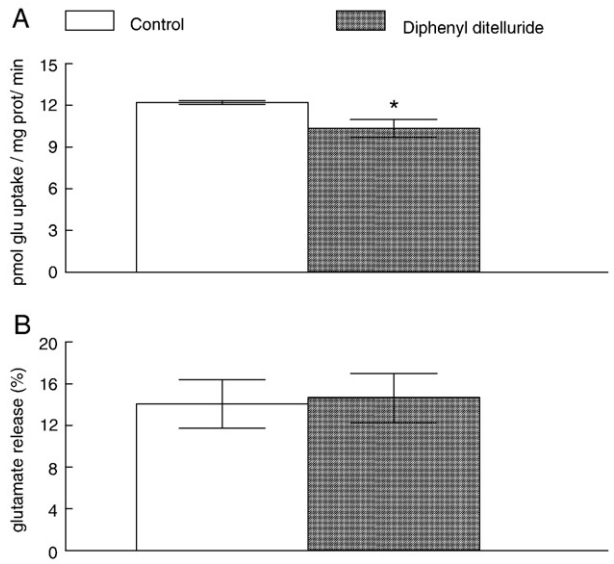

Fig. 3. Evaluation of synaptosomal [^{3}H]glutamate uptake (A) and release (B) of young rats exposed to $(PhTe)_2$ (0.03 mg/kg, experimental group) or canola oil (1 ml/kg, control group) via maternal milk. Results are expressed as mean±S.E.M. for 3 independent experiments performed in triplicate. *$p<0.05$ compared to the control group.

3.2. Object recognition task

Results for recognition memory task are shown in Fig. 2. There were no significant differences among groups in the time spent exploring any of the two identical objects during training ($p>0.05$) or in the time spent exploring the novel object during the LTM test ($p>0.05$). In the STM test, $(PhTe)_2$-exposed group showed a significantly lower time exploring the novel object ($p>0.05$) when compared to the performance to the control group. In addition, control animals showed a significantly higher time exploring the novel object, in the STM test, when compared to the performance of control animals during training ($p<0.05$).

3.3. Synaptosomal [^{3}H]glutamate uptake and release

Results for synaptosomal [^{3}H]glutamate uptake and release are shown in Fig. 3. The [^{3}H]glutamate uptake by synaptosomes was significantly decreased (around 15%) in the $(PhTe)_2$-exposed group when compared to the control group ($p<0.05$) (Fig. 3A). The [^{3}H]glutamate release by synaptosomes from the whole brain of young rats was not different between control and $(PhTe)_2$-exposed groups ($p>0.05$) (Fig. 3B).

3.4. Na^+/K^+ATPase activity

Na^+/K^+ATPase activity was significantly decreased (around 34%) in the $(PhTe)_2$-exposed group when compared to the control group ($p<0.05$) (Fig. 4).

4. Discussion

The present study establishes that young rats presented cognitive impairment after maternal $(PhTe)_2$ exposure, demonstrated by the performance of animals in STM of object recognition memory task. Another finding of this study is that $(PhTe)_2$ significantly inhibited synaptosomal glutamate uptake and cerebral Na^+/K^+ATPase activity in young rats.

Considerable evidence has been accumulated suggesting that inorganic radiotellurium can be transferred to suckling rats in proportions that varied from 2 to 5% of the administered maternal dose (Nishimura et al., 2003). Since a lipophylic form of Te was used in this experimental protocol, one would expect an even higher degree of Te transference from mothers to their litters. Thus, it seems plausible to assume that Te became bio available to suckling rats after exposure of their mothers to $(PhTe)_2$ and may cause behavioral changes in the offspring.

The action of the nervous system and its subtle disruptive functioning caused by xenobiotics could be evaluated through the performance of animals in several behavioral tests (Annau and Cuomo, 1988; Graeff et al., 1998; Lalonde et al., 2003). The object recognition memory task in rodents has been shown to be a very useful experimental tool for assessing changes in neuronal function induced by drugs or genetic modifications. Novel object recognition is a type of non-aversive and non-spatial memory (Puma et al., 1999; Rampon et al., 2000). Evidence in the literature has shown that systemic administration of diphenyl diselenide, $(PhSe)_2$, a selenium compound analogous to $(PhTe)_2$, induces a cognitive enhancer in the object recognition task in mice (Rosa et al., 2003).

In the present study, the behavioral performance of animals suggests that the exposure to $(PhTe)_2$ induces a cognitive impairment in young rats. Our data corroborated with findings reported in the literature that showed a consistent deficit in spatial learning using the water maze task (Widy-Tysziewicz et al., 2002), a persistent neuromotor impairment which is associated with a severe deficit in shock avoidance and lowered sensitivity to noxious stimulus, which in turn would retard the learning of the active avoidance task (Dru et al., 1972) following inorganic Te intoxication. Moreover, maternal exposure to low doses of $(PhTe)_2$ may result in disinhibitory behavior of offspring in the elevated plus maze task (Stangherlin et al., 2006).

In addition, we have obtained persuasive evidence indicating that $(PhTe)_2$ affects a number of neuronal processes and modifies the functionality of the glutamatergic system in vitro and in vivo (Nogueira et al., 2001). 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, novel object recognition memory requires glutamate receptors activation (Rampon et al., 2000). Rodent learning and memory performance have been linked to NMDA-R-dependent forms of synaptic plasticity. NMDA-R-mediated long-term potentiation and long-term depression have been demonstrated in brain regions involved in one-trial object recognition, such as the hippocampus (see Lynch, 2004 for review). Therefore, in this study we investigate if glutamatergic neurotransmission is involved in $(PhTe)_2$-induced behavioral effects. In general, glutamate is the most dominant transmitter across tasks of learning and memory and has

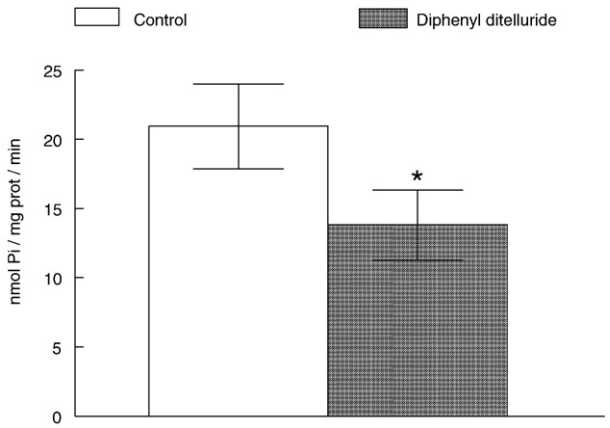

Fig. 4. Determination of cerebral Na^+/K^+ATPase activity of young rats exposed to $(PhTe)_2$ (0.03 mg/kg, experimental group) or canola oil (1 ml/kg, control group) via maternal milk. Results are expressed as mean±S.E.M. for 6–8 independent experiments performed in duplicate. *$p<0.05$ compared to the control group.

been linked to associative processes (Myhrer, 2003). In this context, one candidate for the improvement of memory is a persistent enhancement of glutamate release (Dolphin et al., 1982). Several studies have related facilitated glutamate release with consequent increase in learning (Daisley et al., 1998; Lhullier et al., 2004; Mameli et al., 2005; McGahon et al., 1996). Different from these data, in the present study, synaptosomal glutamate release was not affected by $(PhTe)_2$. On the other hand, $(PhTe)_2$ inhibited glutamate uptake, which might consequently increase the levels of extracellular glutamate. According to the literature data, this event could improve memory in animals. However, the effect observed in the present study was exactly the opposite behavior. One plausible interpretation for our data is that the activation of the glutamatergic neurotransmission may be beneficial or detrimental, depending among other factors on the degree of activation. Slight activation may be beneficial, as observed in memory studies (Izquierdo and Medina, 1997), while over stimulation may be detrimental, as observed in various acute and chronic brain injuries (Maragakis and Rothstein, 2004). Concerning the involvement of the glutamatergic neurotransmission in the impairment of memory induced by $(PhTe)_2$ in young rats, this duality is also present, $(PhTe)_2$ being potentially detrimental or not.

Na^+/K^+ATPase activity seems to be involved in $(PhTe)_2$-induced impairment of recognition memory task. In fact, Na^+/K^+ATPase activity was inhibited in young rats exposed via maternal milk to $(PhTe)_2$. Na^+/K^+ATPase is an enzyme embedded in the cell membrane, responsible for the generation of the membrane potential through the active transport of Na^+ and K^+ ions in the central nervous system necessary to maintain neuronal excitability (Erecinska and Silver, 1994). In this context, the inhibition of Na^+/K^+ATPase activity may be involved in the memory consolidation of step-down inhibitory avoidance in the hippocampus (Wyse et al., 2004). As far as we know it is the first time that the interaction between the recognition memory task and the inhibition of Na^+/K^+ATPase activity has been reported. Moreover, the stimulation of Na^+/K^+ATPase activity was shown to inhibit neurotransmitter release (Vizi and Vyskocil, 1979). Since Na^+/K^+ATPase is crucial for maintaining ionic gradients in neurons and is critically involved in potassium buffering after periods of hyperstimulation (Xiong and Stringer, 2000), it is well acceptable that an inhibition of this enzyme activity might impair neuronal activity and memory storage.

Although the fact that a parallelism of effects was verified between Na^+/K^+ATPase activity and memory, it does not necessarily mean that the reduced activity of this enzyme would be the only cause of the memory impairment observed. Recent studies have also reported that Na^+/K^+ATPase inhibition can lead to memory impairment in the inhibitory avoidance and in the water maze tasks (dos Reis et al., 2002; Sato et al., 2004; Wyse et al., 2004), and to cognitive deficits in degenerative diseases, such as Alzheimer's disease (Hattori et al., 1998; Lehotsky et al., 1999). Therefore, it is tempting to suggest that the inhibition of Na^+/K^+ATPase activity is involved in $(PhTe)_2$-induced impairment of recognition memory.

Taking these data together, another plausible explanation for the impairment of recognition memory induced by $(PhTe)_2$ is the interaction between the glutamatergic system and Na^+/K^+ATPase activity. The postsynaptic actions of glutamate are rapidly terminated by uptake systems located predominantly on astrocytes. Three Na^+-dependent glutamate transporters have been described (Kanner, 1993; Danbolt, 1994), with two of them showing a glial localization (Rothstein et al., 1994, Chaudhry et al., 1995). Glutamate is cotransported with Na^+ into astrocytes and the intracellular concentration of Na^+ rises sufficiently to activate the Na^+/K^+ATPase. Consequently, the inhibition in cerebral Na^+/K^+ATPase activity observed in the present study can be directly related to inhibition of glutamate uptake.

In conclusion, the present study establishes that young rats presented cognitive impairment after maternal exposure to $(PhTe)_2$, demonstrated by the performance of these animals in object recognition memory task. The inhibition of cerebral Na^+/K^+ATPase activity and synaptosomal glutamate uptake could be involved in $(PhTe)_2$-induced cognitive impairment. Additional investigations in specific cerebral structures, such as hippocampus, are necessary to determine the neurochemical mechanisms involved in the effect of $(PhTe)_2$ on learning/memory.

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