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Intrastriatal injection of hypoxanthine impairs memory formation of step-down inhibitory avoidance task in rats

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ABSTRACT

The aim of this study was to investigate the effects of intrastriatal injection of hypoxanthine, the major compound accumulated in Lesch–Nyhan disease, on performance step-down inhibitory avoidance task in the rat. Male adult Wistar rats were divided in two groups: (1) saline-injected and (2) hypoxanthine-injected group. Treated-group received intrastriatal hypoxanthine solution 30min before training session (memory acquisition) or immediately after training session (memory consolidation) or 30 before test session (memory retrieval) on step-down inhibitory avoidance task. Results show that hypoxanthine administration caused significant memory impairment in all periods tested. These results show that intrastriatal hypoxanthine administration provoked memory process impairment of step-down inhibitory avoidance task, an effect that might be related to the cognitive memory alterations in Lesch–Nyhan patients.

1. Introduction

Tissue accumulation of hypoxanthine occurs in patients with Lesch–Nyhan disease, an X-linked hereditary disorder caused by deficiency of hypoxanthine–guanine phosphoribosyltranspherase (HPRT) activity (Nyhan et al., 1965). Affected patients present cognitive deficits, hyperuricemia, spasticity, dystonia and self-mutilation behavior characterized by biting of the lips, tongue and fingers with apparent tissue loss (Jinnah and Friedmann, 2001; Matthews et al., 1999). In addition, studies also show that affected patients present dysfunction of dopamine transmitter system in basal ganglia and a reduction of striatum volume (Jinnah and Friedmann, 2001; Palmour et al., 1989).

Accumulation of hypoxanthine has been proposed to contribute to neurological dysfunction presented in patients with Lesch–Nyhan disease (Brunori, 2001; Kisch et al., 1985; Ma et al., 2001; Visser et al., 2000). In this context, Bavaresco et al. (2007a) demonstrated that intrastriatal injection of hypoxanthine in rats, at the concentration found in Lesch–Nyhan patients, significantly impaired spatial learning/memory in the acquisition phase of the Morris Water Maze and decreased striatal levels of serotonin (5-HT) and 5-hydroxy-indoleacetic acid (5-HIAA). Beside this, Ägren et al. (1983) indicated correlations between higher levels of hypoxanthine in cerebrospinal fluid (CSF) and memory disturbance.

We also showed a decrease on Na^+, K^+–ATPase activity and total radical-trapping antioxidant parameter (TRAP) in striatum, hippocampus and cerebral cortex of rats, as well as an increase in chemiluminescence, in the same cerebral structures, 30min after hypoxanthine infusion in rat striatum (Bavaresco et al., 2007b). In addition, studies show that hypoxanthine may affect neuronal development by enhancing cell proliferation and impairing morphogenesis (Ma et al., 2001).

It has been shown that hypoxanthine infusion impairs memory in task of water Maze in rats (Bavaresco et al., 2007a). Other tasks, like step-down inhibitory avoidance, a conditioned avoidance response task (Rossato et al., 2006) in which declarative or spatial component of a task can be evaluated also are important to evaluate memory/learning (Bavaresco et al., 2007a). Evidence from literature pointed that lesions to central structures could affect the acquisition, consolidation and retrieval memory phases indicating behavioral differences between memory processes in the first few hours or in the following few days, which suggest participation of different mechanisms (Medina et al., 1999). Studies demonstrated that the dorsal striatum is involved in various types of learning/memory such as procedural learning, habit learning, reward-association and emotional learning (Boussaoud and Kermadi, 1997; Ragozzino et al., 2001; Gill and Mizumori, 2006; Ferreira et al., 2008). In this context, Packard et

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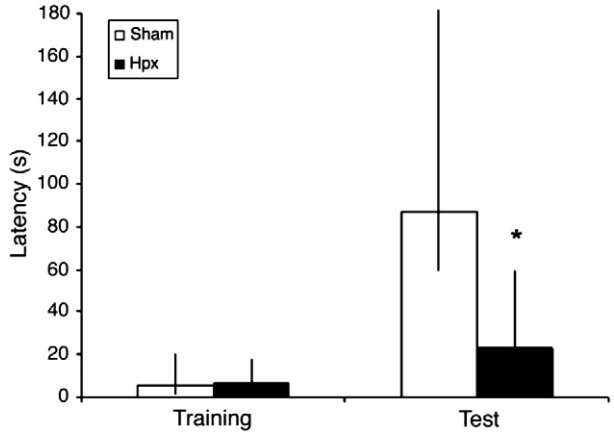

Fig. 1. Effect of intrastriatal hypoxanthine infusion 30 min before training on step-down inhibitory avoidance task. (a) Data are median (interquartile range) of 9–11 animals in each group. *Different from the control group (Mann–Whitney; $p<0.05$).

al. (2006) showed that post-training infusion of metabotropic glutamate receptor (mGluR) antagonist in dorsal striatum impaired retention on step-down inhibitory avoidance task. Moreover, it has been proposed that Na^+, K^+–ATPase activity inhibition (Wyse et al., 2004) and oxidative stress induction (Delwing et al., 2006; Reis et al., 2002) could impair memory formation in rats.

In the present study we investigated the effect of intrastriatal hypoxanthine infusion on step-down inhibitory avoidance task at different periods. The drug was infused into the striatum because patients with this syndrome present characteristic alterations in the basal ganglia (Jinnah and Friedmann, 2001).

2. Materials and methods

2.1. Animals and reagents

Sixty-days-old male Wistar rats were obtained from the Central Animal House of the Department of Biochemistry, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil. Animals were maintained on a 12 h light/dark cycle (lights on from 7 a.m. to 7p.m.) in air-conditioned constant temperature (22°C) colony room, with free access to a 20% (w/w) protein commercial chow and water. Animal care followed the official governmental guidelines in compliance with the Federation of Brazilian Society for Experimental Biology and was approved by Ethics Committee of the Federal University of Rio Grande do Sul, Brazil. All chemicals were purchased from Sigma Chemical Co., St Louis, MO, USA.

2.2. Stereotaxic surgery and cannula placement

Rats were anesthetized with ketamine and xilazine (75 and 10mg/kg ip, respectively) and placed in a rodent stereotaxic apparatus. Under stereotaxic guidance, a 27-gauge stainless cannula (0.9mm O.D.) with an inner needle guide was inserted unilaterally into the right striatum (coordinates relative from bregma: AP, − 0.5mm; ML − 2.5mm; V − 2.5mm from the dura) (Paxinos and Watson, 1986). In our experiments, we utilized a single cannula implanted into the striatum as described by Sánchez-Iglesias et al. (2007). Two days after the surgery, a 30-gauge needle was inserted into the guide cannula in order to inject buffered hypoxanthine (10μM) or vehicle (saline) into the right striatum over a 1min interval. The volume administered (saline or hypoxanthine) was 2μL. Animals were divided into two groups: group 1 (vehicle group), rats that received intrastriatal saline and group 2 (hypoxanthine-treated), rats that received intrastriatal hypoxanthine solution (20pmol/2μL). Hypoxanthine concentration was chosen according to Puig et al. (1989).

2.3. Drug administration procedure

In order to evaluate the effect of hypoxanthine on memory processing phases (acquisition, consolidation and retrieval), drugs were infused into the right striatum at different periods: 30min before training session (memory acquisition), immediately after training session (memory consolidation) and 30min before test session (memory retrieval).

2.4. Behavioral procedures

2.4.1. Step-down inhibitory avoidance task

On the 63rd day of life, animals were subjected to behavioral testing. We used the step-down inhibitory avoidance task since it has been widely used in the study of memory formation (Izquierdo and Medina, 1997; Prado-Alcalá et al., 2003; Wyse et al., 2004); behavioral experiments were conducted between 11h a.m. and 15h p.m.

Animals were subjected to training and test sessions in a step-down inhibitory avoidance task with an interval of 24h in between (Izquierdo and Medina, 1997). This task involves learning not to step-down from a platform in order to avoid a mild foot shock (Izquierdo and Medina, 1997). The task was carried out in an automatically operated, brightly illuminated box. The left extreme of the grid was covered by a 7.0cm wide, 2.5cm high formic platform. Animals were placed on the platform and their latency to step-down, placing their four paws on the grid (42.0×25.0cm grid of parallel 0.1cm caliber stainless steel bars spaced 1.0cm apart), was measured. In test sessions, no foot shock was delivered and step-down latency (with a ceiling of 180s) was used as a measure of memory retention, as described in previous reports (Izquierdo and Medina, 1997; Reis-Lunardelli et al., 2007).

2.5. Statistical analysis

Differences between test and training latency differences on inhibitory avoidance task were assessed by individual (two tailed) Mann–Whitney *U* tests, $p<0.05$ was considered significant. Descriptive statistics data were expressed as median (interval interquartile).

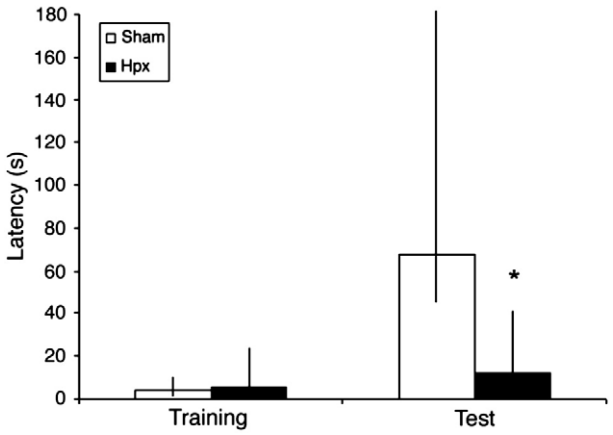

Fig. 2. Effect of intrastriatal hypoxanthine infusion immediately after training on step-down inhibitory avoidance task. (a) Data are median (interquartile range) of 11–12 animals in each group. *Different from the control group (Mann–Whitney; $p<0.05$).

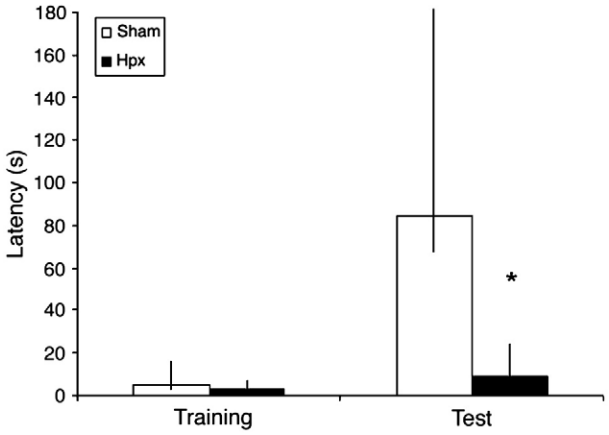

Fig. 3. Effect of intrastriatal hypoxanthine infusion 30 min before test session on step-down inhibitory avoidance task. (a) Data are median (interquartile range) of 8–9 animals in each group. *Different from the control group (Mann–Whitney; $p<0.05$).

All analyses were performed using the Statistical Package for the Social Science (SPSS) software in a PC-compatible computer.

3. Results

3.1. Experiment 1: effect of intrastriatal hypoxanthine infusion 30min before training session on step-down inhibitory avoidance task

Fig. 1 shows the effect of intrastriatal hypoxanthine infusion 30min before training on step-down inhibitory avoidance task. Latency differences in training were not significant among control and hypoxanthine groups in Mann–Whitney *U* test ($U = 45.50$, $p>0.05$). Latency differences in test performance were significant among control and hypoxanthine groups according to Mann–Whitney *U* test ($U = 18.00$, $p<0.05$).

3.2. Experiment 2: effect of intrastriatal hypoxanthine infusion immediately after training session on step-down inhibitory avoidance task

Fig. 2 shows the effect of intrastriatal hypoxanthine infusion immediately after step-down inhibitory avoidance training. Latency differences in training were not significant among control and hypoxanthine groups ($U = 64.00$, $p>0.05$), however there was a latency differences in test performance were significant among control and hypoxanthine groups according to Mann–Whitney *U* test ($U = 26.00$, $p<0.05$).

3.3. Experiment 3: effect of intrastriatal hypoxanthine infusion 30min before test session on step-down inhibitory avoidance task

Fig. 3 illustrates the effect of intrastriatal hypoxanthine infusion 30min before test session on step-down inhibitory avoidance task. Latency differences in training were not significant ($U = 23.00$, $p>0.05$), however, latency differences in test performance were significant among control and hypoxanthine groups according to Mann–Whitney *U* test ($U = 15.00$, $p<0.05$).

4. Discussion

In the present study we investigated the effect of intrastriatal hypoxanthine administration on step-down inhibitory avoidance task. Our results demonstrate that hypoxanthine infusion, at the concentration found in Lesch–Nyhan patients, significantly impaired learning/memory 30min before training or test session as well as immediately after training. This is in agreement with a previous work which showed a significant impairment on learning/memory on the Morris Water Maze task (Bavaresco et al., 2007a). The results obtained in our study probably are not attributed to motor deficits, since we have previously demonstrated that hypoxanthine administration did not alter the open field task (Bavaresco et al., 2007a).

Although the exact mechanism through which hypoxanthine alters learning/memory in rats is still unknown, it has been showed that modulation of Na^+, K^+–ATPase activity is a fundamental mechanism for learning/memory (Brunelli et al., 1997; Reis-Lunardelli et al., 2007; Sato et al., 2004), for long-term potentiation (LTP) induction (Glushchenko and Izvarina, 1997) and learning in distinct models (Brunori, 2001). For instance, bilateral infusion of ouabain, a specific inhibitor of Na^+, K^+–ATPase activity, on chick forebrain causes inhibition on consolidation phase with retention loss persisting at least 24h after training (Gibbs et al., 2003; Sherry and Crowe, 2007) and another study showed Na^+, K^+–ATPase inhibition in rat hippocampus immediately and 6h after training session on inhibitory avoidance task (Wyse et al., 2004).

It has been shown that 30min after intrastriatal hypoxanthine infusion, Na^+, K^+–ATPase activity in striatum, hippocampus and cerebral cortex of rats was significantly decreased (Bavaresco et al., 2007b). Moreover, hypoxanthine *in vitro* significantly inhibits Na^+, K^+–ATPase activity from purified synaptic plasma membrane, suggesting a direct action of these compounds on the enzyme (Bavaresco et al., 2004). It is then conceivable that the inhibitory effect elicited by hypoxanthine on Na^+, K^+–ATPase activity could be one of the mechanism involved on the memory impairment observed.

The induction of oxidative stress caused by hypoxanthine infusion should not be excluded, since oxidative stress is also associated with memory deficits (Bickford et al., 1999; Serrano and Klann, 2004). Evidence showed that hypoxantine induces an increase in reactive oxygen species and/or lipid peroxidation and decreases brain antioxidant capacity (Bavaresco et al., 2005; Bavaresco et al., 2006; Bavaresco et al., 2007b; Beckman et al., 1987). It has been shown that the formation of free radicals by hypoxanthine/xanthine oxidase could contribute to the destruction of blood-brain barrier observed in ischemic brain tissue (Beckman et al., 1987). Moreover, oxidative stress induced by hypoxanthine inhibited Na^+, K^+–ATPase activity in striatum, cerebral cortex and hippocampus of rats (Bavaresco et al., 2004). In fact, it is then possible to suggest that the imbalance between free radical production and antioxidant defenses caused by hypoxanthine administration could lead to memory deficits found in the present study.

The biochemical events involved in memory process could be also modulated by neurotransmitters like serotonin and GABA. In this context, Prado-Alcalá et al. (2003) showed that post-training administration of the 5-HT2 receptor blocker ketanserine produced a memory retention deficit in rats. In addition, Ticku and Burch (1980) demonstrated that hypoxanthine could inhibit benzodiazepine and GABA binding to its receptor-like sites in rat brain membrane. Also elevate extracellular levels of hypoxanthine could bind to benzodiazepine agonist sites in GABA(A) receptor inhibiting memory process (Deutsch et al., 2005; Izquierdo and Medina, 1997; Savić et al., 2005). These evidences give support to the experimental impairment on memory formation obtained in present study.

In conclusion, our results show that intrastriatal hypoxanthine administration provoked memory impairment in rats submitted to step-down inhibitory avoidance task. Considering that Lesch–Nyhan patients present cognitive memory alterations, we suggest that it might be associated to the accumulation of hypoxanthine in brain.

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