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Effects of 3-nitropropionic acid administration on memory and hippocampal lipid peroxidation in sleep-deprived mice

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Abstract

Numerous studies have described memory deficits following sleep deprivation. There is also evidence that the absence of sleep increases brain oxidative stress. The present study investigates the effects of a pro-oxidant agent–3-nitropropionic acid (3-NP)–on hippocampal oxidative stress and passive avoidance performance of sleep-deprived mice. Mice were repeatedly treated i.p. with saline or 5 or 15 mg/kg 3-NP and sleep-deprived for 24 h by the multiple platform method—groups of 4–5 animals placed in water tanks, containing 12 platforms (3 cm in diameter) surrounded by water up to 1 cm beneath the surface or kept in their home cage (control groups). The results showed that: (1) neither a 24 h sleep deprivation period nor 3-NP repeated treatment alone were able to induce memory deficits and increased hippocampal lipid peroxidation; (2) this same protocol of sleep deprivation, combined with 15 mg/kg 3-NP repeated treatment, induced memory deficits and an increase in hippocampal lipid peroxidation. The results support the involvement of hippocampal oxidative stress in the memory deficits induced by sleep deprivation and the hypothesis that normal sleep would prevent oxidative stress.

Keywords: 3-Nitroptropionic acid; Avoidance learning; Memory; Oxidative stress; Sleep deprivation

1. Introduction

There is considerable evidence that sleep plays an important role in memory processes (for review see Siegel, 2001; Walker and Stickgold, 2004). Clinical data have shown that the deprivation of sleep causes deficits in several forms of learning/memory (Cochran et al., 1994; Karni et al., 1994; Fluck et al., 1998; Mednick et al., 2002; Isomae et al., 2003). In addition, numerous studies have demonstrated that sleep deprivation in laboratory animals, among other behavioral alterations (Frussa-Filho et al., 2004; Silva et al., 2004c; Andersen et al., 2005a,b), produces memory deficits in several models, such as avoidance tasks (Bueno et al., 1994; Smith and Kelly, 1998; Silva et al., 2004a,b), Morris water maze task (Smith and Rose, 1996; Youngblood et al., 1999), radial maze task (Smith et al., 1998), contextual fear conditioning (Graves et al., 2003) and object recognition (Palchykova et al., 2006a,b). Further, paradoxical sleep enhancement induced by different procedures improves memory retention in rats tested in the Y-maze discrimination paradigm (Wetzel et al., 2003).

Several research studies on learning and memory in rodents have used the passive avoidance task. In this paradigm, decreased latency to step through in the test session is usually presented by animals with several kinds of cognitive deficits, such as those induced by pharmacological manipulations (Silva et al., 1999; Isomae et al., 2003; Santucci and Shaw, 2003), consequent to neural lesions (Isomae et al., 2003), related to

Abbreviations: 3-NP, 3-nitropropionic acid; MDA, malondialdehyde; SD, sleep deprivation.

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aging (Silva et al., 1996; Yasui et al., 2002) or due to sleep deprivation (Moreira et al., 2003; Silva et al., 2004a,b).

The mechanisms responsible for the occurrence of memory deficits following sleep deprivation are not clearly understood. One of the theories to explain the changes in the cerebral function that follow sleep deprivation proposes that normal sleep would prevent oxidative stress by removing the reactive oxygen species produced during the wake period. In short, sleep deprivation would reduce the antioxidant defenses (Reimund, 1994). Indeed, increases in hypothalamic and thalamic oxidative stress levels were found in sleep-deprived rats (D'Almeida et al., 1998, 2000). Furthermore, Maquet et al. (2002) suggested that the proposed restorative function of sleep might involve the elimination of toxic compounds (e.g. free radicals) and the replenishment of energy stores. Finally, Schulze (2004) proposed that sleep protects excitatory cortical circuits against oxidative damage.

Increased brain oxidative stress seems to have an important role in cognitive impairment caused by normal aging and neurodegenerative diseases. Administration of antioxidant agents has been shown to improve such deficits (Carrillo et al., 1993; Carney et al., 1991; Markesbery, 1997; Small, 1998; Kontush, 2001). In addition, we have recently verified that memory deficits induced by 72 h of sleep deprivation are accompanied by an increase in hippocampal oxidative stress in mice and both effects are abolished by treatment with different antioxidant agents (Silva et al., 2004a).

One of the consequences of an oxidative stress process is an increase in lipid peroxidation. In this respect, lipid peroxidation is caused by an attack of free radicals upon cell membrane lipids (Halliwel and Gutteridge, 1999). Measurement of malondialdehyde (MDA), the most abundant product arising from lipid peroxidation (Kagan, 1988), has been extensively used an index of oxidative stress (Halliwel and Gutteridge, 1999; Gluck et al., 2001; Abílio et al., 2002; Silva et al., 2004a).

The aim of the present study is to extend our previous findings (Silva et al., 2004a) that indicated that sleep deprivation leads to memory impairment via hippocampal oxidative stress. For this purpose, we investigated the effects of a pro-oxidant agent, the 3-nitropropionic acid (3-NP) (Beal et al., 1993), on memory (evaluated by passive avoidance task) and hippocampal oxidative stress (evaluated by lipid peroxidation levels) in mice deprived of sleep for 24 h. It is hypothesized that, if memory impairment due to sleep deprivation acts via a hippocampal lipid peroxidation process, the administration of a pro-oxidant agent should potentiate the effects of sleep deprivation both on memory and hippocampal lipid peroxidation. Specifically, mice that are sleep-deprived for 24 h (not enough to produce memory impairment per se) would present those deficits if pre-treated with 3-NP.

2. Materials and methods

2.1. Subjects

Three-month-old Swiss EPM-M1 male mice (weighing 30–35 g) were housed under conditions of controlled temperature (22–23 °C) and lighting (12 h light, 12 h dark, lights on at 7 a.m.). Food and water were available ad libitum throughout the experiments. Animals used in this study were maintained in accordance with the guidelines of the Committee on Care and Use of Laboratory Animal Resources, National Research Council, USA.

2.2. Drug

3-Nitropropionic acid (Sigma) was dissolved in saline solution and pH adjusted to 7.0 with NaOH. Saline was used as control solution. All substances were given i.p., at a volume of 10 ml/kg of body weight.

2.3. Sleep deprivation procedure

The method of sleep deprivation used was an adaptation of the multiple platform method, originally developed for rats (Nunes and Tufik, 1994). Groups of 4–5 animals were placed in water tanks (41 × 34 × 16.5 cm), containing 12 platforms (3 cm in diameter) each, surrounded by water up to 1 cm beneath the surface, for 24 h. In this method, the animals are capable of moving inside the tank, jumping from one platform to the other. Food and water were made available through a grid placed on top of the water tank. Control animals were maintained in their home cages in the same room.

The described protocol has been shown to prevent the occurrence of both slow-wave and paradoxical sleep under our laboratory conditions (Silva et al., 2004a).

2.4. Passive avoidance task

In passive avoidance experiments, an adaptation of the method previously described for rats (Silva et al., 1996, 1999) was used. The apparatus employed was a two-way shuttle-box provided with a guillotine door placed between the modular testing chambers. One chamber is illuminated by a 40-W bulb, while the other remained in the dark. In the training session, the animals were individually placed in the illuminated chamber, facing away from the guillotine door. When the animal entered the darkened chamber, the door was noiselessly lowered and a 0.5 mA foot shock was applied for 2 s through the grid floor. In the test sessions, the animals were again placed in the illuminated chamber, but no foot shock was applied. Latency to step through was recorded in each session.

2.5. Hippocampus dissection

The animals were killed by decapitation and the brain was removed immediately and washed with ice-cold saline. After that, the hippocampus was quickly dissected and weighed. The whole procedure took no longer than 3 min and brain tissue was always maintained on ice.

2.6. Measurement of hippocampal lipid peroxidation

Immediately after dissection, hippocampi were homogenized in ice-cold 0.1 M phosphate buffer (1:50, w/v). Duplicates of