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Hypomyelination, memory impairment, and blood–brain barrier permeability in a model of sleep apnea

Lenise Jihe Kim[a,*], *Denis Martinez*[a], *Cintia Zappe Fiori*[a], *Diego Baronio*[a], *Nélson Alexandre Kretzmann*[b], *Helena Maria Tannhauser Barros*[c]

[a]*Laboratório Interdisciplinar de Pesquisa em Sono (LIPES-UFRGS), Serviço de Cardiologia, Universidade Federal do Rio Grande do Sul (UFRGS), Hospital de Clínicas de Porto Alegre (HCPA), Ramiro Barcelos, 2350, 90035-903 Porto Alegre, RS, Brazil*

[b]*Centro de Terapia Gênica, UFRGS, HCPA, Brazil*

[c]*Departamento de Ciências da Saúde, Disciplina de Farmacologia, UFCSPA, Brazil*

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ABSTRACT

We investigated the effect of intermittent hypoxia, mimicking sleep apnea, on axonal integrity, blood–brain barrier permeability, and cognitive function of mice. Forty-seven C57BL mice were exposed to intermittent or sham hypoxia, alternating 30 s of progressive hypoxia and 30 s of reoxigenation, during 8 h/day. The axonal integrity in cerebellum was evaluated by transmission electron microscopy. Short- and long-term memories were assessed by novel object recognition test. The levels of endothelin-1 were measured by ELISA. Blood–brain barrier permeability was quantified by Evans Blue dye. After 14 days, animals exposed to intermittent hypoxia showed hypomyelination in cerebellum white matter and higher serum levels of endothelin-1. The short and long-term memories in novel object recognition test was impaired in the group exposed to intermittent hypoxia as compared to controls. Blood–brain barrier permeability was similar between the groups. These results indicated that hypomyelination and impairment of short- and long-term working memories occurred in C57BL mice after 14 days of intermittent hypoxia mimicking sleep apnea.

1. Introduction

Obstructive sleep apnea affects up to one third of the population (Tufik et al., 2010) and is characterized by short, recurrent interruptions in respiratory airflow terminated by an arousal. The breathing instability leads to episodes of intermittent hypoxia (Dempsey et al., 2010).

Patients with sleep apnea show structural and functional disorders of the central nervous system (Lal et al., 2012). Cognitive dysfunctions comprise impairments in attention

*Corresponding author. Fax: +55 51 3022 2282.
E-mail address: lenisekim@gmail.com (L.J. Kim).

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(Sforza et al., 2004), executive function (Lis et al., 2008), and memory (Twigg et al., 2010). In animal models utilizing exposure to intermittent hypoxia to simulate sleep apnea, neuronal death (Zhu et al., 2007) and neurocognitive dysfunctions were observed (Gozal et al., 2001). Our group previously described that cerebellum cells are sensitive to intermittent hypoxia-induced lesions (Baronio et al., 2013).

The mechanisms involved in the association between sleep apnea and cognitive impairment is still unclear. Deficit in executive functioning of children with severe apnea has been linked to decreased neuronal concentration of N-acetyl aspartate, a substrate for lipid and myelin synthesis in oligodendrocytes (Halbower et al., 2006). The brain white matter reduction observed in patients with sleep apnea, including the hypomyelination process in different brain regions (Kim et al., 2013), may play an important role in this relationship. Endothelin-1 has been reported as a key modulator of myelin and axonal damages in animal models of central nervous system injuries (Dos Santos et al., 2007), leading to reactive gliosis and inhibition of neural progenitors proliferation (Li et al., 2010). Moreover, endothelin-1 increases the permeability of the blood–brain barrier in animal models of ischemic stroke (Leung et al., 2009). Sleep apnea increases plasmatic levels of endothelin-1 (Ip et al., 2004). These factors have been studied separately.

We hypothesized that intermittent hypoxia may lead to the impairment in axonal myelination and cognitive performance, as well as to changes in the levels of endothelin-1 and the permeability of the blood-barrier barrier. The present study investigated the effect of 14 days of intermittent hypoxia, mimicking sleep apnea, in serum levels of endothelin-1, blood–brain barrier permeability, axonal integrity, and cognitive function of C57BL mice.

2. Results

2.1. *Endothelin-1 serum levels and blood–brain barrier permeability*

Serum levels of endothelin-1 were significantly higher in the hypoxia group than in the controls (Fig. 1A). No difference in the Evans Blue dye concentrations was observed between groups (Fig. 1B; $P=0.7$; $P=0.8$; $P=0.7$, respectively). No difference between groups was observed in water content of the three regions analyzed (Fig. 1C; $P=0.9$; $P=0.1$; $P=0.6$, respectively).

2.2. *Ultrastructural analysis*

The hypoxia group showed a thinner myelin sheath in cerebellum compared to controls (Fig. 2A and B). Axonal myelin sheath thickness was approximately two-fold larger in control than in hypoxia group animals ($P<0.001$). Axon diameters, however, were similar between control and hypoxia groups ($P=0.9$). The ratio of axon diameter to fiber diameter (*g*-ratio) was significantly higher in animals exposed to hypoxia ($P<0.015$; Fig. 2C), indicating hypomyelination.

2.2.1. *Working and recognition memories*

Fig. 3 shows scores of the animals in the novel object recognition test. Total time exploring both objects was similar between control and hypoxia groups (Fig. 3A). During test 1, the ability to discriminate the two objects was impaired in the hypoxia group, indicated by negative values of discrimination and discrimination index as compared to positive values in the control group (Fig. 3B and E). Hypoxia animals explored the familiar object with more frequency and duration than control group during test 1 (Fig. 3C and D). The information retention and recognition memory were also worse in animals exposed to intermittent hypoxia, indicated by recognition index values lower than 50% (Fig. 3F). The controls presented recognition index above 50%. These results suggest impairment of short-term memory in the hypoxia group.

In the first and second tests, the hypoxia group showed reduced discrimination of novel and familiar objects. These animals spent significantly more time exploring the familiar object, and with more frequency, than the control group (Fig. 3C and D). Discrimination parameters were significantly higher in the control group, indicating the normal preference for novelty (Fig. 3E). Recognition index was also lower in animals exposed to intermittent hypoxia (Fig. 3F), indicating impaired long-term memory.

In the univariate general linear model, controlling for performance in the first test, no significant differences were observed in any variable of the novel object recognition test in the second test ($P>0.05$).

3. Discussion

The findings of the present study comprised the hypomyelination in cerebellum, impairment in short- and long-term memory, and increased levels of endothelin-1 in mice exposed to 14 days of intermittent hypoxia. Furthermore, this is the first study to evaluate the blood–brain permeability in a murine model of sleep apnea.

Cai et al. (2012) reported hypomyelination in a mice model of infantile sleep apnea. They found a reduction in proportion of myelinated fibers in the external capsule, striatum, fornix, and cerebellum. Moreover, the myelin sheath thickness/axon size was significantly lower in the animals exposed to intermittent hypoxia, suggesting a process of demyelination. One noteworthy finding of our study is the extension of the hypomyelination observed after 14 days only of intermittent hypoxia. In studies about axon myelination, chronic intermittent hypoxia protocols are often applied, ranging from 9 days to 4 weeks of exposition (Kanaan et al., 2006; Cai et al., 2012). Although the half-life of some myelin components is around one month in the normal mouse brain (Ando et al., 2003) the response to injury may lead to rapid myelin degeneration. After a crush-induced lesion, myelin disappears in three days (Goodrum et al., 1994) and after an inflammatory insult, myelin disappears in a few days (Jeong et al., 2013).

Imaging techniques have detected brain damage in humans with sleep apnea. These patients show a reduction of the white matter in regions with important neural