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Hippocampal and cerebellar histological changes and their behavioural repercussions caused by brain ischaemic hypoxia experimentally induced by sodium nitrite

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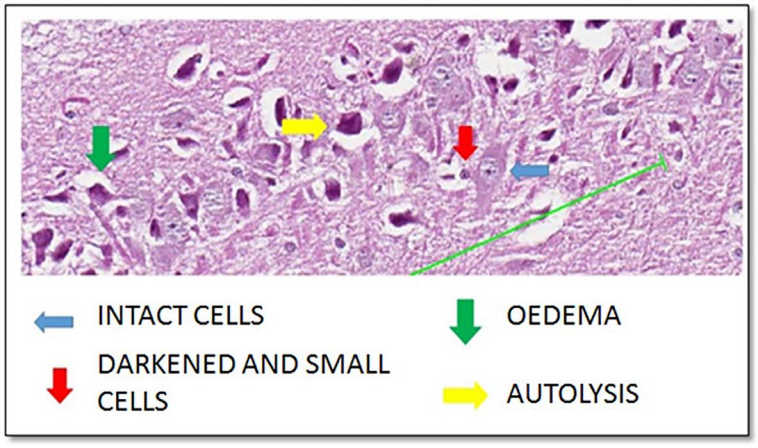
GRAPHICAL ABSTRACT



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ABSTRACT

Introduction: Brain ischaemic hypoxia can produce severe neurological damage that leads to behavioural disorders. This research analysed the hippocampal and cerebellar histological alterations caused by brain ischaemic hypoxia experimentally induced by sodium nitrite ($NaNO_2$) and possible direct repercussions of this hypoxia on behaviour.

Methodology: An experimental study was carried out by administering 60 mg/kg $NaNO_2$ to 10 Wistar rats at 3 months of age for 15 consecutive days. Ten control rats did not receive $NaNO_2$. To assess behavioural repercussions, the animals were evaluated in Open Field, Elevated Plus-Maze (EPM), and Forced Swim tests before and after injury to evaluate locomotion, anxiety, and depression, respectively. Markers of stress were evaluated by measuring the blood levels of cortisol, glucose, cholesterol, and lactate. The presence of hippocampal lesions

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was verified by histologically studying the CA1–CA4 areas. Sections of the cerebellum were also evaluated because Purkinje cells are highly sensitive to ischaemic hypoxia and may serve as markers for this process.
Results: The number of neurons with lesions was significantly higher in animals exposed to $NaNO_2$ in the hippocampus areas CA2, CA3, and CA4. The cerebellum was also very vulnerable to hypoxia, presenting extensive lesion áreas. These results are correlated with the parameters of the anxiety and depression tests.
Conclusion: $NaNO_2$ promoted brain damage due to ischaemic hypoxia in rats. Intoxicated animals showed decreased brain weights; damage in hippocampus and cerebellum; and anxiogenic and depressive behaviour.

1. Introduction

Brain ischaemia is characterized by decreased glucose levels and an oxygen-rich blood supply to the brain and is a predisposing factor for the occurrence of cerebral infarction [1]. Each year, approximately 10 million people survive neurological lesions related to cerebral ischaemic hypoxia [2], but people who survive the early stages of cerebral infarction often suffer from sequelae due to neurological impairment that are associated with a high incidence of behavioural, cognitive and sensorimotor deficits [3,4].

Brain ischaemic hypoxia may be due to several causes, including chemical toxicity. For example, sodium nitrite ($NaNO_2$) is an inorganic salt used to manufacture dyes and treat textile products [5]. Upon reaching the bloodstream, it readily reacts with haemoglobin to form methaemoglobin (MetHb), which may reduce haemoglobin's ability to carry oxygen [6–8]. Furthermore, it induces the formation of reactive oxygen species, which significantly damage neuronal cells [6,7].

Because the central nervous system (CNS) is particularly susceptible to the effects of both hypoxia and free radicals, $NaNO_2$ intoxication can damage this system and may result in extensive neuronal damage in various areas of the brain [8,9]. Depending to the injured areas, behavioural repercussions, such as anxiety and depression, may arise [10]. The hippocampus composes part of the limbic system, which controls various behavioural and cognitive functions [11] and various diseases and ageing may affect hippocampal structure and function [12]. Although the aetiology of hippocampal lesions is diverse, chronic stress [13] and brain ischaemic hypoxia [14] change neuronal electrical properties in this area and influence anxiogenic behaviour [13]. The cerebellum is also a very sensitive region to decrease the supply of oxygen, because in its cortical region predominates gray matter, especially represented by Purkinje cells [15].

Nowadays, many studies have been giving different attention to studies aimed at more accurately detailing the damage caused by hypoxia, without the evaluation related directly to the behaviour being investigated in the same subject affected by this neurological damage. Thus, many researchers end up missing some details in important information to analyse what, in fact, could be reflecting the relationship between hypoxia-induced damage and behaviour is poorly understood. Therefore, this study aimed to analyse the hippocampal and cerebellar histological changes experimentally induced by $NaNO_2$ and their behavioural and biochemical repercussions related to possible stressor events caused by ischaemic hypoxia in the brain.

2. Methodology

2.1. Animals

Wistar rats (n = 20, *Rattus novergicus*) were provided by the Animal Facility of the Natural Sciences Laboratory of the University Centre of Brasília (Centro Universitário de Brasília, UnB) in the Federal District of Brazil. The animals were aged 3 months at the start of the experiment and weighed an average of 200 g. The experimental time period was 1 month. The animals were housed in groups of at most 5 rats per cage maintained in a light-dark cycle of 12 h, and the animals had access to Purina® brand species-appropriate chow and tap water *ad libitum*. The animals were divided in 2 groups of 10 animals each: the control group (CG) consisted of animals that did not receive intervention, whereas $NaNO_2$ was used to induce brain ischaemic hypoxia in the experimental group (EG).

The experimental study was carried out at the Laboratory of Pathology of the University of Brasilia (Universidade de Brasília). All procedures followed the criteria established by the Ethics Committee on Animal Use (Comitê de Ética no Uso Animal – CEUA) of the Biological Sciences Institute of the University of Brasília according to protocol UnBDoc n° 67736/2014.

2.2. Exposure to $NaNO_2$

$NaNO_2$ was used to induce hypoxia. Specifically, $NaNO_2$ transforms haemoglobin to MetHb, which reduces the capacity of the blood to carry oxygen. $NaNO_2$ was dissolved in deionized water and injected intraperitoneally, administered to 3-month-old rats at a dose of 60 mg/kg for 15 days [9]. Animals of the same age that receive only deionized water (without $NaNO_2$ injection) served as controls for our investigations.

2.3. Experimental procedures

During the experiments, behavioural tests were performed, and biometric, biochemical, and histological data were collected. The animals in the CG and EG were weighed, and behavioural tests were performed at 2 time points: 1) when the animals were 3 months old and 2) after 1 month, when the EG animals had already undergone ischaemic hypoxia induced by $NaNO_2$. Behavioural analyses included tests of spontaneous locomotor activity (Open Field test), anxiety (Elevated Plus-Maze − EPM test) and depression (Forced Swim test). At the end of the behavioural experiments, the brains and blood samples were collected for histological and biochemical evaluations, respectively.

2.3.1. Spontaneous locomotor activity

For this test, a 60 × 60 × 35 cm wooden arena was used. The arena's floor was divided into 9 20 × 20 cm quadrants. Each animal was placed in the central quadrant of the arena and tested for a period of 5 min. Each rat's locomotor activity was assessed when it placed all four of its paws into a new quadrant [16]. After each trial, the apparatus was cleaned with alcohol (10% v/v).

2.3.2. Elevated plus maze (EPM)

An EPM maze is a cross-shaped wooden device that is built 50 cm up from the ground with two closed arms (10 × 40 cm) and two open arms (50 × 10 cm) placed in opposition to each other [17]. The apparatus had a 1 cm high transparent acrylic rail around the open arms to prevent the animals from falling off the EPM. Each rat was positioned at the centre of the maze facing one of the closed arms and allowed to explore the maze for 5 min. A single observer recorded the number of entries and the periods of permanence in the open and closed arms. The percentage of entries into the open arms was calculated relative to the total number of entries into both arms, and the period of permanence in the open arms was calculated relative to the total period of the experiment. The anxiolytic effect was defined as an increase in the total periods of entry (or time) into the open arms relative to the total