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Morphological investigation in a rat model of hemic hypoxia

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In this study, we report rat brain morphological changes after sodium nitrite-induced acute hemic hypoxia. Male Wistar rats at the age of four months were intraperitoneally injected with sodium nitrite ($NaNO_2$) at 50 mg/kg. Treated animals were sacrificed at different time intervals (1h and 48h) following the administration. Brain sections were stained using routine histological methods and examined under a light microscope. Vacuolization was observed in corpus callosum of the hypoxic brains. Increased number of the dark epithelial cells of plexus choroideus, macrophages and dark pyramidal neurons in the cortex were the most prominent histopathological changes indicating that $NaNO_2$-induced hypoxia results in rat brain injury.

Key words: hemic hypoxia, sodium nitrite, rat brain morphology

Introduction

Insufficient oxygen or hypoxia produces a great physiological stress inducing cellular responses that result in deleterious effects on certain tissues [7]. Hypoxia is one of the major pathological events causing neuronal cell injury, neurodegeneration and cell death. The brain is the most hypoxic vulnerable of all vertebrate tissues because of its high rate of aerobic metabolism and limited antioxidant defense systems [4]. The pathogenesis of cerebral hypoxia has been associated with a time dependent cascade of molecular events including rapid fall in intracellular adenosine triphosphate with consequent increase in adenosine, anaerobic glycolysis, activation of calcium-stimulated enzymes, mitochondrial dysfunction and formation of reactive oxygen species (ROS) that contribute to the oxidative brain damage [2]. Hemic hypoxia is characterized by reduced oxygen-transport capacity of the blood often due to deficit of hemoglobin and altered transport, binding and delivery of O_2 by the hemoglobin.

Literature data for the effect of hemic hypoxia on brain morphology are insufficient. The present investigation is undertaken to follow up the rat brain morphological changes after $NaNO_2$-induced acute hemic hypoxia.

Materials and Methods

The experiments were carried out on four-month-old male Wistar rats. The animals were maintained in the institute's animal house in standard hard bottom polypropylene cages at 23°C±2°C, 12:12 h light/dark cycle and free access to laboratory chow and tap water throughout the study. Rats (n=10) were subjected to acute hemic hypoxia by a single intraperitoneal injection of $NaNO_2$ at 50 mg/kg body weight (1 ml dosing volume). Treated animals were sacrificed at different time intervals following the administration (1h and 48h) under light anesthesia. The control rats (n=5) were injected with the same volume of distilled water.

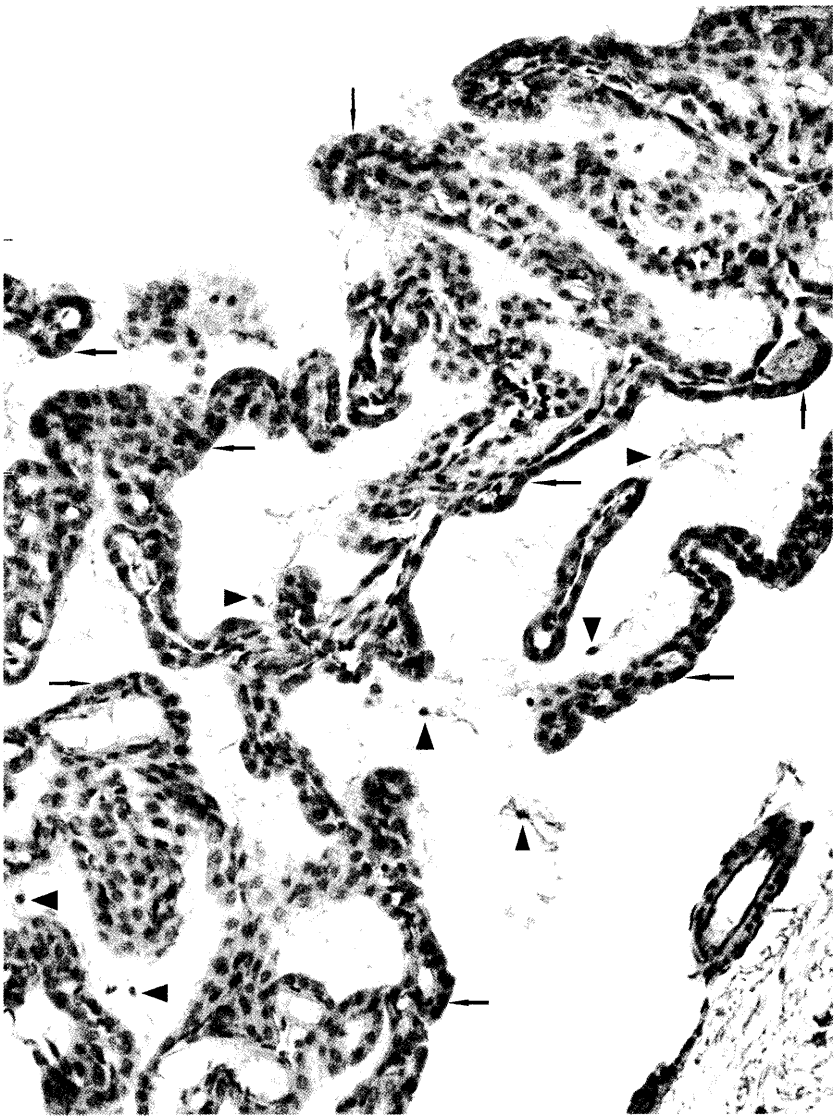

Fig. 1. Light microscopic micrograph of choroid plexus from hypoxic rat brain with dark epithelial cells (⟶) and macrophages (►). HE, x20

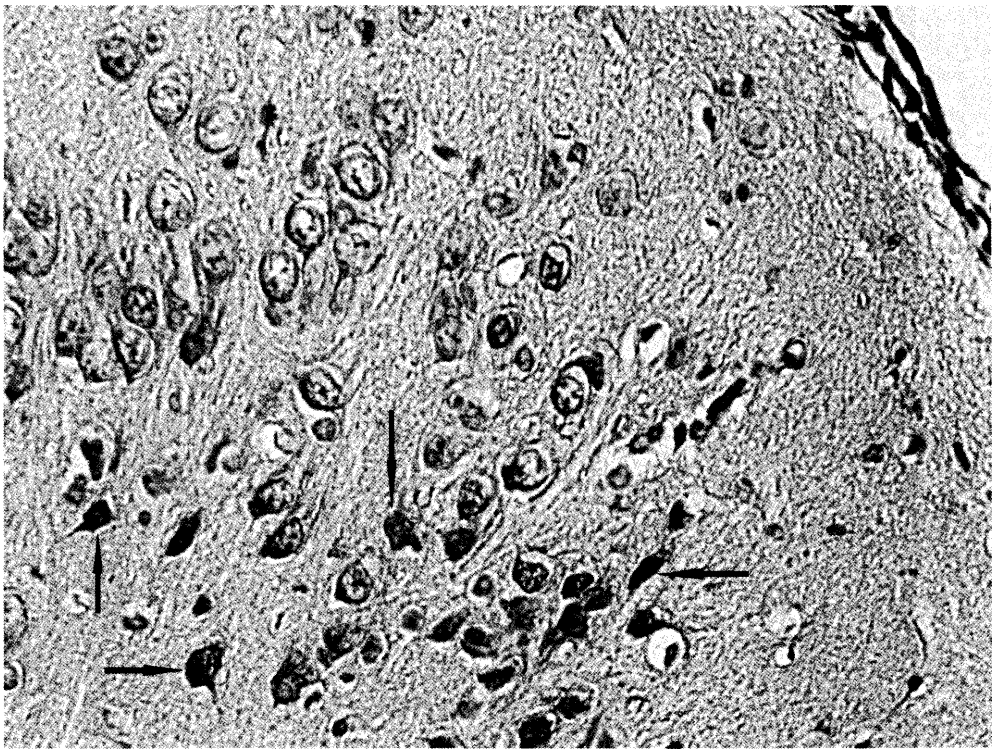

Fig. 2. Light microscopic micrograph of cerebral cortex from hypoxic rat brain with dark pyramidal neurons (➟). HE, x40

Brains were sampled, fixed and embedded in paraffin using routine histological practice. Tissue coronal sections (7 μm) were stained by hematoxylin-eosin *and* examined under light microscope.

The animal experiments were performed in accordance with the animal protection guidelines approved by the Ethics Committee for Experimental Animal Use at IEMPAM, BAS.

Results and Discussion

It is known that brain hypoxia generates a series of biochemical events with several cellular and functional consequences, such as plasma membrane structural damage and delayed cell death [6]. Several experimental models have been used to simulate the human cerebral hypoxia syndrome. In our experiments we have developed a model of acute sodium nitrite-induced hemic hypoxia. Sodium nitrite, an inorganic salt with both harmful and healthful effects, is known as hypoxia inducible agent. In the circulation it causes conversion of hemoglobin to methemoglobin, which is incapable of transporting oxygen to the body's tissues and organs. Thus the oxygen-carrying capacity of the blood is reduced. The administration of $NaNO_2$ in high concentrations may cause brain inflammation, ischemia and impaired cerebral energy [8].

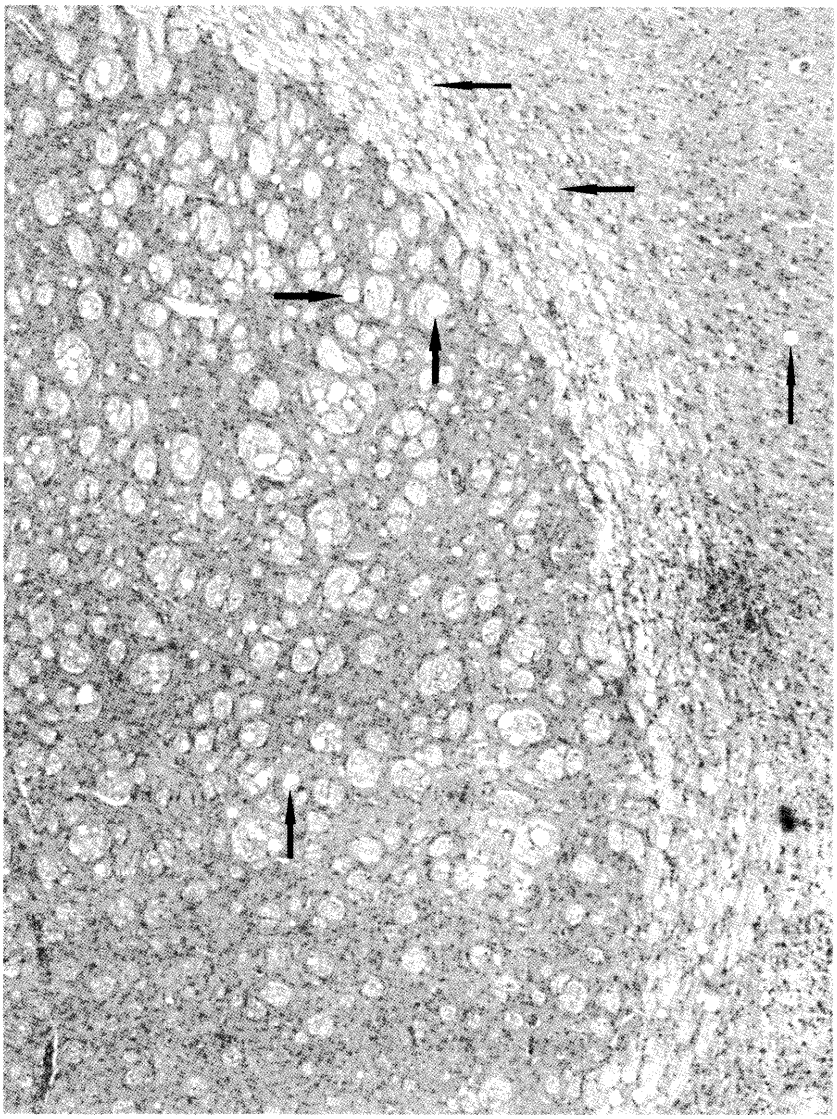

Fig. 3. Light microscopic micrograph of corpus callosum from hypoxic rat brain with vacuoles (➡). HE, x5

One hour following $NaNO_2$-induced hypoxia, we found increased number of the dark epithelial cells of plexus choroideus in the area of the fourth ventricle (Fig. 1). They are generally considered to be modified ependymal cells with epithelial cell characteristics and referred to as choroidal epithelial cells. Moreover, the number of macrophages was also increased. In the cerebral cortex, a large number of dark pyramidal neurons was observed (Fig. 2). These changes may be indicative of impaired secretory transport and represent a compensatory mechanism against hypoxic injury. Further transmission electron microscopic studies would elucidate the observed histopathological findings.

Morphological changes in the rat brain after $NaNO_2$-induced hypoxia are also documented by Zaidi [9]. Degenerating nerve cell bodies with pyknotic nuclei and vacuolar spaces in dentate gyrus, as well thinning of hippocampal and dentate gyrus blades have been demonstrated at the first hour following $NaNO_2$ injection. Extensive cerebellar Purkinje cell damage is also documented with swollen, autolytic and shrunken cells [8]. It is concluded that Purkinje cells are very vulnerable to hypoxic insult and $NaNO_2$-induced hypoxia results in a significant excitotoxic degeneration of these cells.

In our experiments vacuolization was observed 48 hours after $NaNO_2$ injection, mainly in corpus callosum and the deeper structures beneath it (Fig. 3). Changes in the vascular endothelium were also found though they would be characterized in detail by electron microscopy. The underlying mechanism of $NaNO_2$ influence on the brain is not clearly understood. Nitrite is known to cause free radical generation [5], as it can stimulate oxidation of ferrous ions in oxyhemoglobin to form methemoglobin as well as various ROS [1, 3].

In conclusion, the results of the present work are indicative of rat brain injury following acute hemic hypoxia. Future studies would elucidate the morphological changes at later stages, as well their reversibility.

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