Original Article

SODIUM NITRITE-INDUCED HYPOXIC INJURY IN RAT HIPPOCAMPUS

Zeenat Fatima Zaidi

ABSTRACT

Objective: To explore the effect of sodium nitrite-induced hypoxia on the hippocampus and the dentate gyrus in adult rats.

***Methodology*:** Adult male albino rats, weighing 180-200 gm were divided into two groups and treated as follows: Group I: served as control and received normal saline, Group II: served as hypoxic rats and received sodium nitrite (75 mg/kg) subcutaneously. One hour after sodium nitrite injection, rats were decapitated. The brains were removed and placed overnight in fixative containing 10% formalin. These were paraffin- embedded for hematoxylin and eosin staining and cut at 5 µ in the coronal plane. Sections passing through bregma level -2.8 to -3.3 mm were used to count the neurons in the CA1, CA2, CA3, and CA4 subsectors and the dentate gyrus. Round, clear and medium or large neurons with distinct nucleus were counted. Cells with darkly stained shrunken nuclei and cells with fragmented nuclei were excluded from the count.

***Results*:** Cytological examination of hypoxic brains depicted degeneration in hippocampus and dentate gyrus. Cell density was comparatively lesser in all the sub regions of hippocampus i.e. CA1-CA4 and Dg in the hypoxic brains. The degeneration was evident by presence of pyknotic nuclei, darkly stained cells, cells with condensed nuclei, as well as vacuolated spaces. The changes were more marked in CA3, CA4 and dentate gyrus.

***Conclusion*:** It is concluded that pyramidal neurons of the hippocampus and granular neurons of the dentate gyrus are very vulnerable to hypoxia and show regional differences in their vulnerability.

KEY WORDS: Hypoxia, Sodium nitrite, Hippocampus, Pyramidal cells, Dentate gyrus, Granule cells.

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INTRODUCTION

The brain is highly sensitive to hypoxia especially the parts that are crucial for cognitive function. These brain structures are closely associated with memory and learning, and probably related changes in synaptic transmission.[1] Whereas, prolonged hypoxia (e.g. suffocation) results in death; shorter intervals of hypoxia may result in lesser degrees of brain damage. The effects of hypoxia are especially severe in the hippocampus. Individuals who survive a hypoxic episode often sustain hippocampal

damage and anterograde amnesia. It has been known that during hypoxia electrical activity disappears especially early in the hippocampus and the cerebellum.[2] Cessation of neuronal firing is a prominent feature in the vulnerable CA1 region of hippocampus, evident within some tens of seconds of hypoxia. Hypoxia suppresses both ongoing and synaptically-evoked firing of CA1 neuron in hippocampal slice *in vitro*. Severe and chronic hypoxia has demonstrated neuronal death in the deep and peripheral structures like CA3, CA4 of areas of hippocampus; dentate gyrus, thalamus, cerebral cortex and striatum.[3] Chronic hydrocephalus–induced hypoxia elicited a profound increase in vascular endothelial growth factor receptor 2 (VEGFR-2) in hippocampus that corresponded to increased blood vessel density.[4] Rapid and severe volume loss in the hippocampus has been reported after neonatal hypoxia-ischemia.[5]

The aim of the present study was to investigate the effect of sodium nitrite-induced hypoxia on the hippocampus and dentate gyrus in adult rats.

METHODOLOGY

Chemicals: All drugs and chemicals used in the present study were of high analytical grade and were obtained from Sigma-Aldrich Co. Sodium nitrite was dissolved in normal saline.

Animals: Adult male albino rats, weighing 180-200 gm obtained from the animal house of King Saud University, were used in this study. They were fed with a standard laboratory diet and tap water ad libitum and housed in cages (ten rats per cage). All animals were kept at standardized laboratory conditions (25±5ÚC, 55±5% humidity, and a 12 h light/dark cycle). One week after acclimatization, the animals were fasted for three hours prior to subcutaneous injection of sodium nitrite. All experiments were carried out according to recommendations of Experimental Animals Ethics Committee, King Saud University which is matched with international ethics for handling of experimental animals. The dose of sodium nitrite used in the current study matched with those in the literature.[6]

Brain Tissue Preparation: Animals were divided into two groups and were treated as follows: Group I (n= 2 rats): served as control and received normal saline, Group II (n= 6 rats) served as hypoxic rats and received sodium nitrite (75 mg/kg). One hour after sodium nitrite injection, rats were decapitated. The brains were removed and placed overnight in fixative containing 10% formalin. Paraffin-embedded brain tissue blocks were cut serially into coronal slices of 5 µ thickness and stained with hematoxylin and eosin (H and E) staining. Quantitative analysis of neuron cell bodies in the pyramidal cell layer of hippocampus and granule cell layer of dentate gyrus was performed

Sections passing through bregma level -2.8 to -3.3 mm were used to count the neurons in the CA1, CA2, CA3, and CA4 subsectors and the dentate gyrus. Round, clear and medium or large neurons with distinct nucleus were counted. Cells with darkly stained shrunken nuclei and cells with fragmented nuclei were excluded from the count. All data were expressed as mean±SEM. The differences in cell count data were analyzed using Student t-test. A $P < 0.05$ was accepted as significant.

RESULTS

Cytological examination of hematoxylin and eosin stained sections of hypoxic rats, depicted degeneration in all the subsectors of hippocampus i.e. CA1-CA4 and dentate gyrus (Figure-1) The degeneration was evident by the thinning of hippocampal and dentate gyrus blades (Figure 2). It was evident by presence of pyknotic nuclei, darkly stained cells, cells with condensed nuclei, as well as vacuolated spaces (Figure-3,4). Cell density was comparatively lesser in all the sub regions of hippocampus i.e. CA1-CA4 and Dg in the hypoxic brains. The changes were more marked in CA3, CA4 and dentate gyrus. The cell counts are depicted in Table-I.

DISCUSSION

Brain cells are extremely vulnerable to fluctuations in the extracellular environment,

Table-I: Effect of sodium nitrite-induced hypoxia on cell density in hippocampus and dentate gyrus in rats.

Group	*CA1*	*CA2*	*CA3*	*CA4*	*Dgub*	*Dglb*
Control	135.5±7.5	50.0±5.0	73.0±6.0	76.5±1.5	323±4.2	307±6.0
Hypoxic	89±9.2*	34.33±2.4*	37.5±2.9*	27.5±2.09*	142.8±15.8*	129.6±11.5*

Values are mean±SEM. mP<0.05 different from control group by Student's t test.

including ischemic stress, trauma and infectious challenges, and can begin to die within five minutes after oxygen supply cuts off. Symptoms of mild cerebral hypoxia include inattentiveness, poor judgment, memory loss, and a decrease in motor coordination. When hypoxia lasts for longer periods of time, it can cause cognitive impairment, coma, seizures, and even brain death. Brain tissue damage results from hypoxia associated with ischemia in conditions such as stroke.[7]

Hypoxia induces morphological changes and permanent neuronal damage in the rat brain.[8] The extent of hypoxic damage is a function of degree and duration of exposure.[9] The brain tissue induces protective mechanisms within minutes, when challenged by stressors or substrate deprivation, to limit the damage.[10] Despite the many coping mechanisms that have evolved to combat the effects of hypoxia, once the tissue has been damaged it is at risk of triggering apoptosis. While the brain is particularly susceptible, within minutes of a stroke or heart failure, delicate neural tissue is already beginning to shut down as the processes that initially protected the brain begin to fail and irreversible damage ensues.

Severe damage to the hippocampus results in profound difficulties in forming new memories (anterograde amnesia), and also affects memories formed before the damage (retrograde amnesia). In Alzheimer's disease the hippocampus is one of the first regions of the brain to suffer damage; memory problems and disorientation appear among the first symptoms.

The hippocampus contains high levels of mineralocorticoid receptors, which make it more vulnerable to stress compared to most

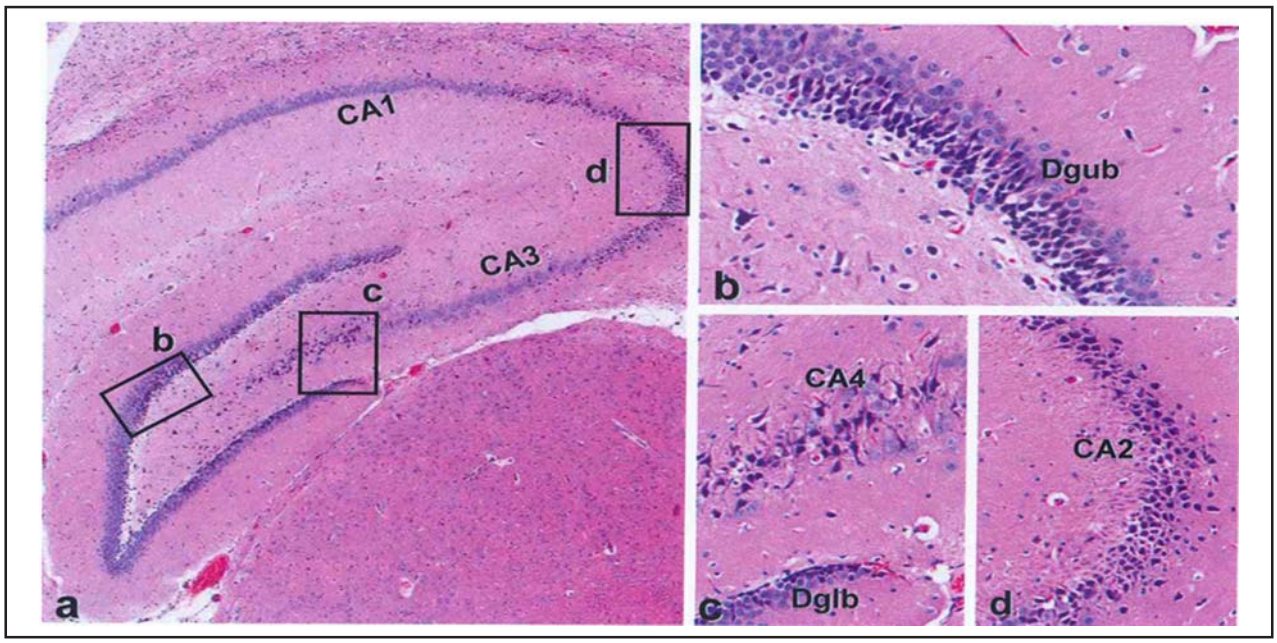

Fig-1: Section through hippocampus of hypoxic rat. (a) Showing regions with damaged cells *X 10*. Insets b, c and d show degenerating nerve cell bodies in upper blade of dentate gyrus (Dgub), CA4 and lower blade of dentate gyrus (Dglb), and CA2 respectively *X 40.*

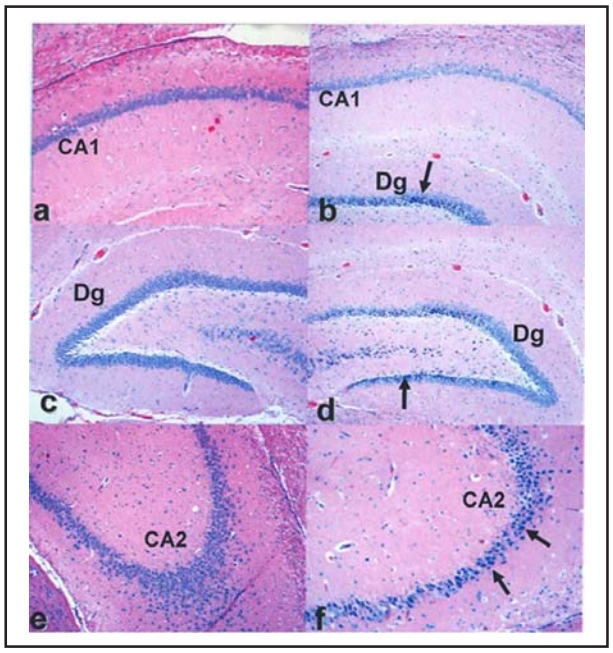

Fig-2: Section through hippocampus of hypoxic rat (b, d, f) showing thinning of hippocampal & dentate gyrus blades compared to normal rat (a, c, e) *X 40.*

other brain areas.[11] There is evidence that humans who have experienced severe, long-lasting traumatic stress show atrophy of the hippocampus, more than other parts of the brain. These effects show up in post-traumatic stress

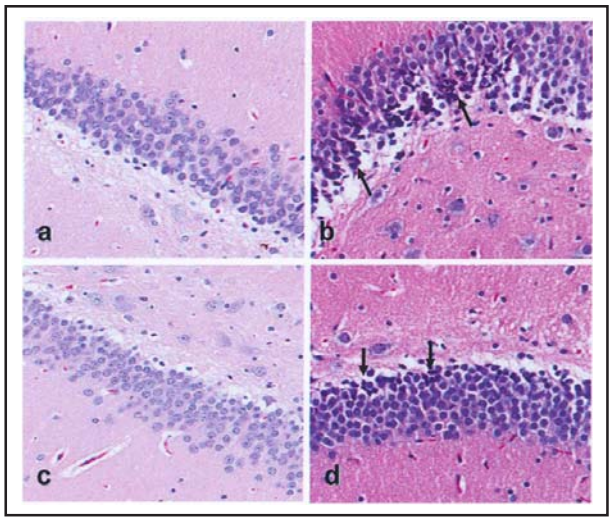

Fig-4: Sections through dentate gyrus of normal and hypoxic rats. a and c show upper and lower blade respectively from the normal rat. b and d are from the upper and lower blade respectively from the hypoxic rat showing degenerating nerve cell bodies marked with pyknotic nuclei (arrows) and vacuolar spaces *X 40.*

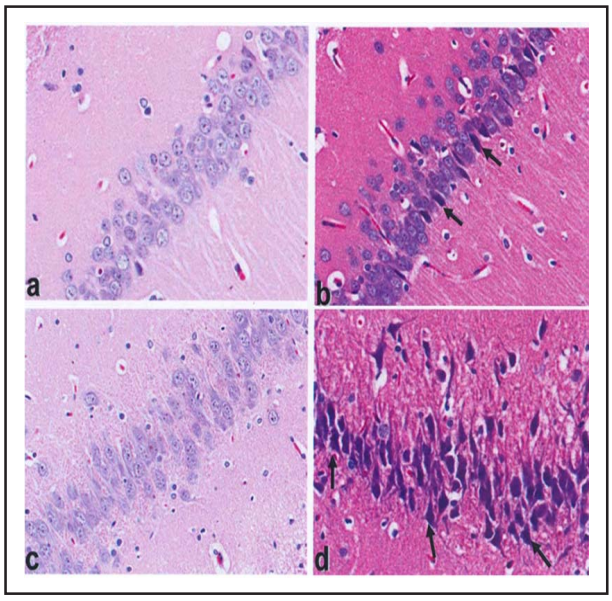

Fig-3: Section through hippocampus of normal and hypoxic rats. a and c show CA1 and CA3 regions respectively from the normal rat. b and d are from the CA1 and CA3 regions respectively from the hypoxic rat showing degenerating nerve cell bodies marked with pyknotic nuclei (arrows) *X 40*

disorder, and may contribute to the hippocampal atrophy reported in schizophrenia and severe depression.[12] The hippocampus is often the focus of epileptic seizures: hippocampal sclerosis is the most commonly visible type of tissue damage in temporal lobe epilepsy.[13] Many reports have found reductions in the size of the hippocampus in schizophrenic subjects.[14] Obstructive sleep apnea (OSA) patients presenting with depressive symptoms showed damage in the bilateral hippocampus.[15] Children with congenital central hypoventilation syndrome (CCHS), a genetic disorder characterized by diminished drive to breathe during sleep and impaired CO2 sensitivity, show brain structural and functional changes on magnetic resonance imaging (MRI) scans, with impaired responses in specific hippocampal region associated with memory, mood, and indirectly, autonomic regulation.[16] Apoptotic death of the neurons in CA3, dentate gyrus and lateral thalamus of the new born rats has been reported after exposure to hypoxial ischemia.[17] Repeated daily restraint stress caused apical dendrites of CA3 pyramidal neurons to atrophy.[18]

It is evident from many studies that there are differences between hippocampal areas in their vulnerability to global ischemia. The CA1 and CA3 regions of the hippocampus markedly differ in their susceptibility to hypoxia[19] hypobaric hypoxia[20] and more particularly to the intermittent hypoxia that characterizes sleep apnea.[21]

The pyramidal neurons in area CA1 and some neurons in the hilus are most vulnerable, whereas most of the CA3 pyramidal neurons survive and the granular cells in dentate gyrus are resistant to ischemic damage.[22] Our results clearly indicated that hippocampus and dentate gyrus are severely damaged by chemical hypoxia and neurons in CA3, CA4 are more vulnerable compared to the neurons in the CA1. These observations were in line with earlier reports that hypoxia damaged the hippocampal neurons.[3,13] In contrary to our results some earlier reports have shown more damage to CA1 after hypoxia.[13] Exposure to hyperbaric hypoxia showed damage to neurons in CA3, but there were no visible morphological alteration in CA2 and the dentate gyrus regions.[20] However, in a study on guinea pig hippocampal slices, the ventral dentate gyrus granule cells (upper blade) were more susceptible to hypoxia than the dorsal ones (lower blade), indicating regional differences of neuronal susceptibility to hypoxia in the dentate gyrus.[23]

High vulnerability of CA3 and CA4 subsectors compared to CA1 in this study could be related to high metabolic rate in CA3 than CA1.[24] It has already been reported in Mongolian gerbils that CA3 pyramidal layer had statistically more significant vasculature in terms of number of capillary vessels, their average diameter, and exchange and flow surfaces than CA1. Paradoxically, the CA1 layer was denser, but lesser vasculature than in the CA3 layer.[24] Another possibility for the neuronal damage was the excitatory toxicity due to glutamate release. It was known that the hippocampal neurons express more glutamate receptors[25] especially in CA1 and CA3 subfields of hippocampus[26] which may be a contributing factor for neuronal damage in CA1 and CA3. It has been reported that during hypoxia the glutamate and aspartate levels rise dramatically, which cause excitotoxicity or disrupt the major excitatory input to the hippocampus, that normally protects the CA1 layer from hypoxic damage.[27]

Limitations of the study: The sample size is small. Hence further studies with bigger sample size are suggested to confirm our observations.

CONCLUSION

Sodium nitrite-induced hypoxia damaged the hippocampal pyramidal and the dentate gyrus granular neurons. Both in hippocampus and dentate gyrus, regional differences in neuronal vulnerability to hypoxia were evident. It is suggested that the long lasting cognitive deficits due to hypoxia may be due to mass neuronal damage/loss in the hippocampus and the dentate gyrus.

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