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nNOS expression in the brain of rats after burn and the effect of the ACE inhibitor captopril

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ABSTRACT

Objective: To investigate the role of endogenous neuronal nitric oxide synthase (nNOS) on brain injury after burn and the effects of the captopril.

Methods: Wistar albino rats (200–250 g) were exposed on the dorsal surface to 90 °C (burn) or 25 °C (sham) water for 10 s. The ACE group was treated with intraperitoneal 10 mg/kg captopril immediately after burn and this treatment was repeated twice daily. At the end of the 24 h brain samples were taken. nNOS was studied in brain areas by immunohistochemistry.

Results: There was no difference between the cerebellar and hypothalamic areas the nNOS expression of all groups. nNOS expression increased in the frontal cortex, striatum and midbrain in the burn group compared to the control group. In the frontal cortex, nNOS expression significantly decreased after ACE inhibitor treatment ($p < 0.05$). The striatal nNOS of the ACE group significantly increased when compared to the control group ($p = 0.001$). In the midbrain of the animals, nNOS decreased in the ACE group. Hippocampal nNOS expression did not change after burn and significantly increased after ACE inhibitor therapy ($p < 0.05$).

Conclusions: Our data showed that the pathophysiological events following burn appear to be related to an acute inflammatory reaction which is associated with nNOS in the frontal cortex, striatum and midbrain, and captopril treatment abrogates the nNOS response in the frontal cortex and midbrain.

1. Introduction

Thermal injury causes pathophysiological changes at the site of injury as well as in distant organs. After burn, changes in the morphology and metabolism of brain tissue have been reported [1,2].

The response of the central nervous system (CNS) to traumatic injury involves molecular mechanisms such as the expression of multiple-signaling molecules, including neurohormones, transcription factors, cellular stress proteins, and pro-inflammatory cytokines. Nitric oxide (NO) is a major messenger molecule which regulates immune function and blood vessel dilatation, acts as a neurotransmitter in the

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central and peripheral nervous system, and is involved in wound healing [3–5].

In a thermal injury model, it was shown that thermal injury altered NO production in vivo and it has been suggested that the hyperdynamic cardiovascular and hypermetabolic responses after thermal injury could be a result of the autocrine and paracrine effects of NO formed locally within the tissues and generated by inflammatory cells [6].

Becker et al. [7] reported that, following a 30% total body surface area burn in rats, there was a significant increase in urinary nitrate excretion which persisted for the 8 days of the experiment.

The renin–angiotensin system (RAS) participates in homeostatic processes that include fluid and electrolyte balance and pressor effects by central and peripheral renin angiotensin systems. In the CNS, neurons and fibers display angiotensin-like immunoreactivity and have a widespread distribution, particularly sites known to regulate the neuroendocrine, cardiovascular, and autonomic systems [8]. By activating angiotensin II (AT II) receptors, circulating angiotensin II may act on central cardiovascular centers via angiotensinergic neurons, providing a link between peripheral and central angiotensin II systems. These peptides in RAS increase blood pressure, water intake, and anterior and posterior pituitary hormone release and may modify memory and learning [9].

Neuronal nitric oxide synthase (nNOS) expression is elevated in some brain regions after burn. In view of the compelling evidence for increased NOS expression and disturbed brain NO metabolism in burn, we hypothesized that the concomitant increase in NOS expression corresponded with elevated brain tissue AT2 expression in burn. We further considered that if this hypothesis is true, then captopril, an angiotensin-converting enzyme (ACE) inhibitor, therapy should ameliorate this process.

The purpose of this study was to investigate how the brain is affected during thermal injury in the acute phase and to assess the effect of RAS via captopril, an ACE inhibitor, on nNOS expression during thermal injury in rats. In doing so, we determined the expression patterns of nNOS in different areas of the brain.

2. Materials and methods

2.1. Animals and laboratory

Wistar albino rats of both sexes, weighing 200–250 g, were obtained from the Marmara University School of Medicine's Animal House. Approval of the Marmara University Ethical Committee for Experimental Animals was obtained before the experiments were conducted. The rats were kept in a temperature-controlled room (22 ± 1 °C) with 12-h:12-h light and dark cycles and fed standard rat chow and water ad libitum and were fasted for 12 h before the experiments.

2.2. Burn model

Groups of rats were anesthetized with ether inhalation and the dorsal hair was clipped. The exposed skin on the back was immersed in 90 °C water bath for 10 s. This procedure has been shown to result in a burn involving 30% of the total body surface area [10]. Sham control rats, which served as controls, were anesthetized, shaved, and exposed to 25 °C water bath for 10 s. After sham or burn, the rats were resuscitated with physiological saline solution (10 ml/kg subcutaneously on the hind limb). Each group consisted of 8 rats. ACE inhibitor Captopril (10 mg/kg, in saline) or saline was given intraperitoneally to rats immediately after the burn and the injections were repeated twice a day.

2.3. nNOS analysis

The rats were decapitated and brain samples were collected 24 h after injury. The brain samples were sent to Baskent University Istanbul Hospital Pathology Laboratory in 10% formaldehyde tamponate solution. Samples from tissues of the frontal cortex, striatum, hippocampus, hypothalamus, cerebellum, and midbrain were taken to undergo routine tissue processing and paraffin blocks were prepared. Tissue sections of 3 μm from paraffin blocks were treated with immunohistochemical rabbit monoclonal [EP1855Y] nNOS (neuronal) stain. The positively stained cell counts were recorded with 20 times amplification under light microscopy Olympos. An experienced pathologist who was unaware of the treatment conditions performed the immunohistochemical assessments.

2.4. Statistical analysis

All data are expressed as means ± s.e.m. The effect of burn or drug treatment on burn was tested using *Kruskal–Wallis* followed by *Dunn's multiple comparison test*. Statistical significance was accepted where $p < 0.05$.

3. Results

Six brain areas were examined: the frontal cortex, striatum, hippocampus, hypothalamus, cerebellum, and midbrain.

In the frontal cortex, the nNOS expression of the burn group was found to be significantly higher than that of the control group. The nNOS expression in the frontal cortex was significantly decreased after ACE inhibitor treatment and found to be lower when compared to the burn group ($p < 0.05$) and there was no difference between the control and ACE group in frontal cortex nNOS expressions (Figs. 1 and 2).

The striatal nNOS expression of the burn group increased significantly compared to that of the control group and the nNOS expression of the ACE group also significantly increased compared to the control group ($p = 0.001$). There was no statistically significant difference between the burn and ACE groups (Figs. 1 and 3).

The hippocampal nNOS expression significantly increased after ACE inhibitor therapy when compared to the burn group that did not undergo drug therapy ($p < 0.05$). There was no significant difference between the hippocampal nNOS expressions of the other groups (Fig. 1).