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Overpressure blast-wave induced brain injury elevates oxidative stress in the hypothalamus and catecholamine biosynthesis in the rat adrenal medulla

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HIGHLIGHTS

- A single OBI was performed in rats to assess the activation of hypothalamic sympatho-adrenal-medullary axis.
- Adrenal medullary catecholamine synthetic enzymes and NPY protein expression as well as plasma NE were elevated.
- NADPH oxidase activity was increased in the hypothalamus.
- TH protein was elevated in the NTS.

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ABSTRACT

Explosive overpressure brain injury (OBI) impacts the lives of both military and civilian population. We hypothesize that a single exposure to OBI results in increased hypothalamic expression of oxidative stress and activation of the sympatho-adrenal medullary axis. Since a key component of blast-induced organ injury is the primary overpressure wave, we assessed selective biochemical markers of autonomic function and oxidative stress in male Sprague Dawley rats subjected to head-directed overpressure insult. Rats were subjected to single head-directed OBI with a 358 kPa peak overpressure at the target. Control rats were exposed to just noise signal being placed at ~2 m distance from the shock tube nozzle. Sympathetic nervous system activation of the adrenal medullae (AM) was evaluated at 6 h following blast injury by assessing the expression of catecholamine biosynthesizing enzymes, tyrosine hydroxylase (TH), dopamine-β hydroxylase (DβH), neuropeptide Y (NPY) along with plasma norepinephrine (NE). TH, DβH and NPY expression increased 20%, 25%, and 91% respectively, following OBI ($P < 0.05$). Plasma NE was also significantly elevated by 23% ($P < 0.05$) following OBI. OBI significantly elevated TH (49%, $P < 0.05$) in the nucleus tractus solitarius (NTS) of the brain stem while AT1 receptor expression and NADPH oxidase activity, a marker of oxidative stress, was elevated in the hypothalamus following OBI. Collectively, the increased levels of TH, DβH and NPY expression in the rat AM, elevated TH in NTS along with increased plasma NE suggest that single OBI exposure results in increased sympathoexcitation. The mechanism may involve the elevated AT1 receptor expression and NADPH oxidase levels in the hypothalamus. Taken together, such effects may be important factors contributing to pathology of brain injury and autonomic dysfunction associated with the clinical profile of patients following OBI.

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1. Introduction

Blast-related traumatic brain injury (TBI) poses a significant concern for military personnel engaged or veterans previously deployed in war zones [3]. The pathophysiology of blast exposure is complex and uniquely different than typical civilian traumatic brain injury as a result of physical trauma or impact to the head. Blast exposure in military situations has various components including: (a) blast overpressure wave-induced injury; (b) secondary injury caused by debris fragments; (c) tertiary injury due to the acceleration or deceleration of the body or body parts due to blast wind or surrounding object; (d) toxic gas, flash burns or intense heat induced bodily injury; and (e) blast noise [3]. Because blast overpressure wave is a primary component of blast-induced organ injury, we previously described an overpressure brain injury (OBI) procedure in rodents using a shock-tube device that can be used as a model for the blast overpressure wave experienced by military personnel [26]. The major effects of OBI have been generally attributed to its external physical impact on the organs, causing internal mechanical damage. The resulting pathophysiological effects include elevated heart rate, blood pressure, respiratory rate, and body temperature [10], as well as cognitive impairment and post-traumatic stress disorder related traits [28].

One recognized pathophysiological consequence of blunt-force-mediated TBI is disruption of autonomic function, resulting in augmented sympathoactivation, but the precise nature of this disruption is not completely understood. Sympathoactivation contributes to systemic stress and cardiovascular complications [3,10]. It is known that TBI is associated with activation of the hypothalamic-pituitary-adrenal (HPA) axis [9]. Another critical participant in the stress response is the hypothalamic sympatho-adrenal-medullary axis [17]. Whether TBI also activates this axis is unknown. Blast induced TBI increases reactive oxygen species (ROS), such as superoxide radicals and nitric oxide [6,29]. In addition, we previously demonstrated that AT1 receptor expression and NADPH oxidase activity in hypothalamus contribute to the activation of the hypothalamic mediated sympathetic outflow [7,8]. Collectively, these data suggest that OBI may stimulate hypothalamic AT1 receptors and NADPH oxidase leading to increased ROS with subsequent activation of the sympatho-adrenal-medullary system.

The nucleus tractus solitarius (NTS) is another brain nucleus that participates in the stimulation of sympatho-adrenal-medullary system following stress [13,17]. The NTS serves as the primary autonomic center that receives viscerosensory inputs from the spinal cord, and cranial nerves project to the NTS through the sensory trigeminal tract. Noradrenergic neurons within the A2 cell group of the NTS, in turn project to the hypothalamus [17].

The sympatho-adrenal-medullary axis leads to marked activation of the AM and sympathetic ganglia characterized by elevated activity of the catecholamine biosynthesizing enzymes such as TH and DβH, resulting in a rise in circulating epinephrine and NE [23]. TH is the rate-limiting step in catecholamine biosynthesis as it catalyzes the hydroxylation of tyrosine to dopamine [20], while DβH catalyzes the conversion of dopamine to NE. In addition to catecholamines, neuropeptide Y (NPY) is synthesized in the AM and is co-released with epinephrine and NE [12,27]. The aforementioned factors, TH, DβH, and NPY are considered the biomarkers of sympathetic nervous system (SNS) activity.

The present study tests the hypothesis that a single exposure to OBI results in increased hypothalamic expression of oxidative stress and activation of the sympatho-adrenal medullary axis. To this end, we measured NADPH oxidase activity and AT1 mRNA expression in the hypothalamus, TH protein expression in the NTS, TH, DβH, and NPY protein expression in the AM as well as plasma NE following a mild-moderate blast overpressure wave.

2. Materials and methods

2.1. Animals

Three month old (250–300 g) male Sprague-Dawley (Harlan Laboratories, Indianapolis, IN) rats were randomly assigned to one of two experimental groups: (1) control ($n = 4$) and (2) brain injury (TBI) induced by blast overpressure wave ($n = 4$). Animals were maintained on a 12:12 h light–dark cycle and provided food and water ad libitum for 2 weeks prior to the experimental protocol. Experiments were conducted according to the Guiding Principles in the Care and Use of Laboratory Animals, and procedures were approved by the local Institutional Animal Care and Use Committee.

2.2. Experimental protocol

Animals in the TBI group were exposed to a single head-directed overpressure blast injury (OBI). A compressed air-driven shock tube was used to expose the TBI rats to a supra-atmospheric wave of air pressure [26]. The tube was separated into two sections: high-pressure (driver) and low-pressure (driven) separated by a metal diaphragm. In these experiments 0.05 mm thick stainless steel diaphragms were used to generate the high pressure shockwaves. The diaphragms were scored with two diagonal and perpendicular lines thus the diaphragms will break away along the score lines without any shrapnel generated. The ratio of driver versus driven section lengths was equal to 15. The driver section was initialized to a pressure of 5170 kPa and maintained at ambient conditions. The diaphragm rupture was initiated by an internal cutter that led to the sudden exposure of a low pressure gas to a gas at a significantly higher pressure resulting in the formation of a shock wave. The blast pressure data were acquired using PCB piezoelectric blast pressure transducers and LabView 8.2 software. A National Instruments 500,000 samples/s data acquisition card was used to acquire data from multiple channels.

Under isoflurane anesthesia rats were placed into a dense Polyethylene holder exposing only their head (body-armored setup) from the exit nozzle of the shock tube with the head positioned directly under the exit nozzle at distance of 5 cm, as described previously [26]. Rats were subjected to a blast wave with a mean peak overpressure of 358 kPa, and a positive pressure phase duration of approximately 10 ms. The noise control animals were positioned 2 m from the exit nozzle thus preventing the rats from experiencing the pressure wave during the diaphragm rupture. The noise duration is about 10 ms with a peak noise level at 100–105 dB. In addition, a control group of naïve animals were included for some western blot analyses.

During the recovery period, the rats are awake and able to walk normally without vocalization of pain. Based on our observation and previous study the above overpressure exposure produces a mild to moderate brain injury. Rats show significant and punctate neurodegeneration as evidenced by increased silver staining in subcortical regions, hippocampus and subthalamic nuclei [26]. Using neurodegeneration cupric silver staining, we observed significant diffused neuronal injury in caudal diencephalon as well as subthalamicus [25].

2.3. Tissue preparation

Six hours following exposure, animals were over-anesthetized with pentobarbital (120 mg/kg ip) and the AM, hypothalamus, and