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Autonomic responses to blast overpressure can be elicited by exclusively exposing the ear in rats

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ABSTRACT

Blast overpressure has become an increasing cause of brain injuries in both military and civilian populations. Though blast's direct effects on the cochlea and vestibular organs are active areas of study, little attention has been given to the ear's contribution to the overall spectrum of blast injury. Acute autonomic responses to blast exposure, including bradycardia and hypotension, can cause hypoxia and contribute to blast-induced neurotrauma. Existing literature suggests that these autonomic responses are elicited through blast impacting the thorax and lungs. We hypothesize that the unprotected ear also provides a vulnerable locus for blast to cause autonomic responses. We designed a blast generator that delivers controlled overpressure waves into the ear canal without impacting surrounding tissues in order to study the ear's specific contribution to blast injury. Anesthetized adult rats' left ears were exposed to a single blast wave ranging from 0 to 110 PSI (0–758 kPa). Blast exposed rats exhibited decreased heart rates and blood pressures with increased blast intensity, similar to results gathered using shock tubes and whole-body exposure in the literature. While rats exposed to blasts below 50 PSI (345 kPa) exhibited increased respiratory rate with increased blast intensity, some rats exposed to blasts higher than 50 PSI (345 kPa) stopped breathing immediately and ultimately died. These autonomic responses were significantly reduced in vagally denervated rats, again similar to whole-body exposure literature. These results support the hypothesis that the unprotected ear contributes to the autonomic responses to blast.

1. Introduction

Blast, such as that produced by explosive devices, has become a frequent cause of injury in both military and civilian populations (Cernak and Noble-Haeusslein, 2010; Taber et al., 2006). Injuries from blast wave overpressure are a source of uncertainty in that they often lack clinically apparent signs and can be insidious, only presenting themselves after other injuries have been managed (Bass et al., 2012; Burgess et al., 2010; Lemonick, 2011; Phillips, 1986). Detection, management, and prevention of blast injury has become an active area of study in blast literature, and more clinical diagnoses can be attributed to the effect of blast overpressure than in the past (Burgess et al., 2010; Svetlov et al., 2009).

Traumatic brain injury, including blast-induced neurotrauma (BINT), is the “signature injury” of U.S. soldiers involved in recent conflicts in Iraq and Afghanistan (Huber et al., 2013; Mac Donald et al., 2014; Moss et al., 2009; Pham et al., 2015). Blast exposure

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may cause BINT through multiple pathways such as direct passage of the blast wave through the skull, compression of the torso resulting in transfer of the blast wave's kinetic energy to the brain via hydraulic oscillations within the vasculature, and hypoxia through over-activation of the parasympathetic nervous system (Cernak, 2010; Cernak et al., 2001; Courtney and Courtney, 2009; Long et al., 2009; Moss et al., 2009; Simard et al., 2014). The parasympathetic response, specifically, is hypothesized to be a result of hyperinflation of the lungs stimulating alveolar juxtacapillary J-receptors innervated by vagal fibers, leading to apnea then tachypnea, bradycardia, and hypotension, which in turn lead to a Bezold-Jarish reflex further deepening bradycardia and hypotension (Cernak, 2010; Krohn et al., 1942; Zucker, 1986; Zuckerman, 1940). Another proposed mechanism suggests that the foramina of the skull (e.g., the acoustic meatus, optic canal, nasal cavity, or foramen magnum) can provide a conduit for the blast wave to enter the cranial vault in addition to the established mechanisms mentioned above (Hicks et al., 2010; Ropper, 2011; Sundaramurthy et al., 2012). The advanced combat helmet, the current helmet of the U.S. Army, uses layers of Kevlar and a foam suspension to protect the skull from penetrating and blunt-force injuries, but leaves the ears, eyes, nose, and mouth all exposed to the surrounding air, permitting pressure from a blast to interact (Meaney et al., 2014; Moore et al., 2009). Nonetheless, soldiers frequently wear little to no ear protection, citing a necessity for situational awareness through unhindered sound localization (Abel, 2008; Brown et al., 2015; Clasing and Casali, 2014; Jones and Pearson, 2016). A perforated eardrum is the most frequently reported blast injury (Cernak and Noble-Haeusslein, 2010; Choi, 2012; Darley and Kellman, 2010; DePalma et al., 2005; Gan et al., 2016; Garth, 1994; Helling, 2004; Katz et al., 1989; Kronenberg et al., 1993; Mayorga, 1997; Patterson and Hamernik, 1997; Phillips, 1986). We hypothesize that energy from blast overpressure could enter the unprotected ear canal, traverse these soft tissues into the cranial vault, and directly impact the brain.

In this study, we tested the hypothesis that the ear provides a vulnerable locus for blast energy to impact the brain while causing acute autonomic responses typically observed in whole-body paradigms of blast exposure (Guy et al., 1998; Krohn et al., 1942; Sawdon et al., 2002). To do so, we developed a blast generator that delivers precisely controlled blast overpressure waves targeted to a small area of tissue, with minimal impact on the surrounding tissues (Fig. 1A). Though placing an animal in a “shock tube” is widely accepted to be the most accurate laboratory model of primary blast, i.e. the shockwave effect of blast (Alay et al., 2017; Needham et al., 2015), there is not a feasible way to prevent the blast wave from causing flexion of the skull and compression of the torso that would introduce confounding factors in our study of the ear's role. Instead, our blast generator produces an overpressure wave similar to that of shock tube literature, but isolates the wave's impact to the ear and minimizes exposure of the rest of the body, including the lungs. This approach allows us to specifically study the ear's role in blast injury, minimizing confounding factors encountered when blast is delivered over the whole animal (Cernak, 2005; Mediavilla Varas et al., 2011; Xiong et al., 2013; Yarnell et al., 2013).

Blast-induced injuries to the organs of the inner ear are active areas of study using whole-body exposure paradigms (Akin and Murnane, 2011; Chandler and Edmondt, 1997; Chen et al., 2013; Cho et al., 2013; Choi, 2012; Cohen et al., 2002; Darley and Kellman, 2010; Dougherty et al., 2013; Fausti et al., 2009; Gan et al., 2016; Garth, 1994; Helling, 2004; Hoffer et al., 2010; Jagade et al., 2008; Kerr and Byrne, 1975; Niwa et al., 2016; Scherer et al., 2011; Singh and Ahluwalia†, 1968; Teter et al., 1970; Tungsinmunkong et al., 2007). However, to the best of our knowledge, this is the first study of the ear's specific contribution to the systemic autonomic responses induced by blast injury.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley (SD) rats (Harlan Sprague–Dawley, Indianapolis, IN) weighing 250–500 g were used for this experiment. In total, 31 rats were used in this study; 22 Rats were used for the autonomic response measurements, and 9 rats were used for the subsequent vagal denervation study. Rats were assigned to exposure intensities randomly. All procedures were performed in accordance with NIH guidelines and approved by the Institutional Animal Care and Use Committee at the University of Mississippi Medical Center.

2.2. Blast wave generator and calibration

A modified high-power airgun was used as the basis for the blast generator (Fig. 1A). All seals and connectors were replaced and/or reinforced with high-quality o-rings and poly-tetra-fluoro-ethylene (PTFE) tape to minimize unwanted pressure escape and increase repeatability. An M1-3KPSI digital pressure gauge (Crystal Engineering Corp. San Luis Obispo, CA) was connected to the air canister to measure input air pressure at a resolution of 0.1 PSI (0.7 kPa). The muzzle of the blast generator was threaded and an aircraft-grade aluminum t-fitting (Eaton, Dublin, Ireland) was affixed for use as a sensor bung. A high-frequency integrated-circuit-piezoelectric (ICP) pressure sensor connected to a signal conditioner (102B04, 480C02, PCB Piezotronics, Depew, NY) was installed in the sensor bung. The blast generator was installed on a stereotaxic frame (David Kopf Instruments, Tujunga, CA) using an adjustable multi-arm instrument holder. A 3.0 mm stainless steel speculum was attached to the instrument holder and held even with the level of the muzzle, leaving open space for excess blast wind to escape. For calibration, a second arm was attached holding another high-frequency ICP pressure sensor and adjusted such that the sensor was in the position of the rat's tympanic membrane (2.5 mm from the tip of the speculum). The blast generator was charged to 500 PSI (3447 kPa) input pressure, allowed to come to equilibrium, and then activated with both the bung sensor and the output sensor recording at 500 kHz. After the blast, the generator was given time to return to equilibrium and then activated again. This was repeated until the air canister was depleted (about 45 blasts). This process was repeated twice to verify repeatability. Each of the waveforms produced by the blast generator were then analyzed for rise time, total positive pressure time, maximum pressure, and return to baseline pressure. The blast pressures were linearly related to the input pressure as well as the bung pressure. Thus, desired blast pressure was achieved by adjusting the input pressure and was verified by the bung pressure.

2.3. Blast exposure

Rats were maintained under 2% inhaled isoflurane anesthesia. Before blast exposure, a photograph of the left and right tympanic membranes was captured using a digital macroview otoscope (Welch-Allyn, Skaneateles Falls, NY) and evaluated for intactness and lack of erythema or effusion. If either factor was present, the rat was not used for this study. A PE-50 polyethylene catheter (Intramedic by BD, Franklin Lakes, NJ) was implanted in the left femoral artery and connected to a disposable transducer (Argon Medical, Athens, TX) for blood pressure monitoring. If a pulsatile waveform was recorded, the catheterization was deemed successful. If such a

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