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Research article

Effect of acute restraint stress in a polytrauma rat model

Françoise Arnaud[a,b,*], Georgina Pappas[a], Ye Chen[a], Eric Maudlin-Jeronimo[a], Richard McCarron[a,b]

[a] Naval Medical Research Center, NeuroTrauma Department, Silver Spring, 20910, MD, United States
[b] Uniformed Services University of Health Sciences, Department of Surgery, Bethesda, 20889 MD, United States

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ABSTRACT

Introduction: A stressful environment may contribute to poor outcomes after TBI. The current study evaluates the impact of acute stress in a polytrauma rat model.
Methods: Rats were stressed by a 45-minute immobilization period before instrumentation under ketamine (t1). Polytrauma was produced by blast overpressure and controlled hemorrhage (t2). Rats were euthanized immediately after a 3 h simulated Medevac-transport time (t3) or after 72 h post-trauma (t4). Corticosterone, ACTH, and ACTH receptor gene expression were measured at these time points. Physiological parameters were monitored throughout the study.
Results: HR was higher in stressed compared to unstressed animals at t1. Corticosterone and ACTH levels were similar for all conditions at t1 and t2; ACTH and corticosterone became elevated in all groups at t3 and at t4, respectively. The ACTH receptor gene expression trended towards higher values at t4 for the stressed animals whether being injured or not. Survival after injury was 83% in both unstressed and stressed animals.
Conclusion: Overall, corticosterone was not significantly affected following acute stress in ketamine-anesthetized rats. Early mortality was primarily due to polytrauma and change in the animal's biochemical parameters appeared at t4 post trauma. The findings indicate that ketamine-anesthesia and/or surgery may have overshadowed the effect of the initial stress.

1. Introduction

Traumatic events such as combat for military members or assaults for civilians are often preceded or followed by physical and psychological stresses that trigger a fight-or-flight response as an acute and healthy response for survival. This response is regulated by the sympathetic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis to maintain a homeostatic balance, both physically and cognitively. When mammals are exposed to stressful situations, the HPA axis becomes activated and stress response mediators such as catecholamines and steroids are systemically released via blood circulation. These stress-response mediators caused increased heart rate and changes in blood pressure and cardiac rhythm, along with increased energy metabolism requirements. All of these physiological changes are beneficial for the organism to overcome and survive stressful situations. Once the stress is over, the HPA axis is able to "turn off" the systemic stress response through negative feedback mechanisms and the organism's physiology returns to homeostatic levels.

However, in the presence of multiple injuries such as traumatic brain injury (TBI) combined with hemorrhage (HS) or other soft tissue injury (i.e., polytrauma), the inability to alleviate the initial stressor could result in negative regulations and alter endocrine, biochemical, metabolic, and physiological responses [1–4]. The negative physiological impact of this stressful situation could affect health outcomes contributing to psychopathology such as post-traumatic stress disorder (PTSD) [5]. Therefore, assessing the impact of restraint stress on the HPA response in the presence of physical injuries, should be addressed in trauma models [1]. Analgesic or sedatives agents such as opioids or non-narcotics are also known to affect the neurohormonal system [6] and in turn can influence results from experimental studies evaluating physiological variables and stress response. These types of medications are needed for experimental studies to provide humane care for research animals [7], it is also important to consider the potential confounding effects that they have on experimental outcomes.

Abbreviations: TBI, traumatic brain injury; HS, hemorrhage; PTSD, post-traumatic stress disorder; ACTH, adrenocorticotropic hormone; EBV, Estimated Blood Volume; MAP, mean arterial pressure; MC2R, ACTH receptor melanocortin-2; Hsp, heat shock protein; MANOVA, multiple analysis of variance; CBC, complete blood cell; WBC, white blood cell; Hb, hemoglobin; Hct, hematocrit; TIVA, total intravenous anesthesia

* Corresponding author at: Naval Medical Research Center (NMRC), Operational and Undersea Medicine, NeuroTrauma Department, 503 Robert Grant Ave, Silver Spring, MD 20910-7500, United States.
E-mail address: Francoise.arnaud.ctr@mail.mil (F. Arnaud).

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The goal of this study was to evaluate the effects of stress and polytrauma using a rat polytrauma model. Immobilization stress, in contrast to a chronic stress model (e.g., fear repetition), was selected to simulate a combat situation of an isolated warfighter unable to escape, prior to potential injuries [5]. Our hypothesis was that a pre-injury stressful situation would cause significant changes in the acute endocrine, physiological and metabolic response immediately after injury and after a 3 day post-injury survival compared to unstressed controls. The primary outcomes were blood corticosterone, adrenocorticotropic hormone (ACTH), ACTH receptor gene expression, blood glucose level and heart rate (HR) [8].

2. Methods

2.1. Animals

The study protocol was reviewed and approved by the Walter Reed Army Institute of Research/Naval Medical Research Center Institutional Animal Care and Use Committee in compliance with all applicable Federal regulations governing the protection of animals in research.

Upon arrival, 10–12 weeks old male Sprague Dawley rats (Charles River, Laboratories, Wilmington, MA), were single-housed in a colony that was maintained on a 12:12 light-dark cycle (lights on from 6:00 to 18:00). A 5-day quarantine period allowed time for acclimation to the environment and handling by the investigators prior to any experimental manipulations. Rats were fasted for ~16 h before the first experimental intervention with water provided *ad libitum* [9,10]. Fasting minimized the individual response to the anesthetic drugs (Animal care and use committee, John Hopkins, http://web.jhu.edu/animalcare/procedures/survival-rodents.html#vendors). During the post-op recovery time, the animals were returned to their cages and monitored twice daily for adverse health effects.

2.1.1. Experimental design

Fig. 1 is a schematic representation of the experimental design. Briefly, the experimentation started at the same time (9am) with the 45 min immobilization stress phase (stress group) or not immobilized (no-stress group) and then all animals were anesthetized. t1 was defined as the end of instrumentation (catheter and monitoring devices in place for the blood collection), t2 was defined as the end of injury (blast and hemorrhage while under anesthesia). Medevac ground transport time was simulated by a 3 h observation period post-trauma (t3) during which the animals were allowed to recover from anesthesia.

The animals (388 ± 27 g, 335–487 g) were distributed into four treatment groups: no-stress + no-injury, no-stress + injury, stress + no-injury, and stress + injury. These groups were further subdivided according to the experimental end points: t3 or t4 referring to euthanasia immediately after simulated Medevac transportation or at 72 h, respectively. This resulted in a distribution of animals into the following groups: no-stress + no-injury (n = 9 for t3 and n = 14 for t4), no-stress + injury (n = 7 for t3 and n = 11 for t4), stress + no-injury (n = 10 for t3 and n = 13 for t4), and stress + injury (n = 8 for t3 and n = 10 for t4). A fifth group of rats (sham, n = 12) were instrumented only for blood samples and then immediately euthanized. A last control group of rats (naïve, n = 9) were anesthetized and blood was taken by cardiac puncture. Blood samples were collected under sedation before euthanasia at t1, t2, and t3, or at t4.

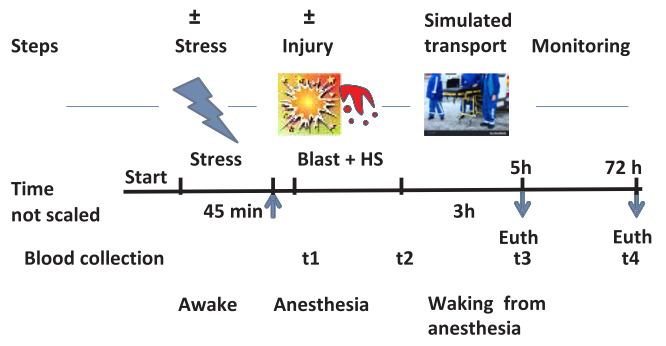

Fig. 1. Experiment design evaluating the effect of stress, polytrauma and simulated transport.

2.1.2. Stress

The rats in the stress groups were placed in a supine position in a plastic cone (DecapiCone, Braintree Scientific, Inc. Braintree, MA) with a breathing aperture placed inside a rat restrainer. These rats were immobilized for 45 min [11] while unstressed rats remained in their cages.

2.1.3. Anesthesia

Animals in all groups were initially anesthetized with a mixture of ketamine (70 mg/kg) and acepromazine (3 mg/kg; IP) followed by buprenorphine (0.05 mg/kg; SC), a pre-emptive analgesia, that was administered before instrumentation. The animals were placed on a heating pad (Kent Scientific) to maintain normothermia at 37 °C and received an additional dose of ketamine (20 mg/kg; IP) after 15 min and subsequent doses (10 mg/kg) as needed to maintain a surgical plane of anesthesia (no response to toe pinch for surgical catheter placement). A final dose of ketamine (10 mg/kg; IP) was administered before transportation to the blast room. During the entire study, animals were breathing spontaneously (FiO_2 = 0.21, room air).

2.1.4. Catheter placement

The femoral artery was catheterized using PE50 tubing (Intramedic, Becton Dickenson, Sparks, MD) to collect blood, infuse saline, and measure blood pressure. Blood was collected by cardiac puncture from the naïve animals. After fluid administration (t2), the catheter was removed, the femoral artery ligated and surgical incision closed prior to anesthetic recovery.

2.1.5. Blast injury (TBI)

The TBI was produced by exposure to blast overpressure generated in a laboratory shock tube [12,13]. Following the blast exposure, the animal was returned to the surgical suite for the remainder of the experiment. Only rats assigned to the injury groups received the blast wave exposure; uninjured rats were transported to the blast room where they remained for an equivalent duration of time as injured rats, but were not exposed to a blast wave.

2.1.6. Hemorrhage

After TBI, animals in the injury groups also underwent a 30% Estimated Blood Volume (EBV) controlled hemorrhage (HS) over a 5-minute period.

(see supplemental material for more detailed description of the injury)

2.1.7. Fluid administration

Animals in the no-injury groups received ~2 ml of 0.9% saline (immediately after blood collection). The animals in the injury groups were resuscitated 15 min after hemorrhage over a period of 5 min using 0.9% saline at twice the volume of blood loss.

2.1.8. Physiology measurements

Mean arterial pressure (MAP) was monitored using a pressure monitoring device (BPA, Micro-Med Inc, Louisville, KY) linked to the arterial catheter. Temperature (Temp), peripheral oxygen saturation (SpO_2), and heart rate (HR) were recorded via a rectal probe, and a clip pulse-type sensor placed on the animal's paw (Physiosuite, Kent Scientific Corp., Litchfield, CT).