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Impaired blood pressure compensation following hemorrhage in conscious obese Zucker rats

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ABSTRACT

Aims: Hemorrhagic shock leads to a higher risk of mortality and morbidity in obese patients, however the mechanisms for these outcomes are unclear. We hypothesized that following severe hemorrhage, blood pressure control in conscious obese Zucker rats (OZ) is impaired.
Main methods: Experiments were performed in conscious lean Zucker rats (LZ) and OZ. Blood pressure, heart rate, cardiac output, total peripheral resistance (TPR), plasma renin activity (PRA), plasma antidiuretic hormone (ADH), and blood gasses were measured before and after severe hemorrhage (35% of the total blood volume).
Key findings: Basal blood pressure, cardiac output, TPR, PRA, and ADH levels were not different between LZ and OZ. Compared to LZ, OZ exhibited impaired baroreflex control of heart rate and showed higher levels of vascular adrenergic tone. One hour after the hemorrhage, LZ and OZ exhibited similar decreases in cardiac output. However, blood pressure, heart rate, TPR, PRA, and ADH levels were lower in OZ than in LZ.
Significance: These results indicate that conscious OZ has impaired blood pressure compensation after hemorrhage due to a blunted increase in TPR. This is due at least in part to an impaired regulation of vasoconstrictor hormones. To our knowledge, the current study is the first to demonstrate that hemodynamic responses and associated hormone secretion are impaired in a conscious obese model.

Introduction

Severe blood loss leads to hemodynamic instability, organ failure, and an increased inflammatory response (Schmid-Schonbein and Hugli, 2005; Sharma et al., 2008). Maintenance of arterial pressure is essential to adequately perfuse vital organs. Critical physiological responses to hemorrhage-nduced hypovolemia are rapid increases in both sympathetic nerve activity via baroreflex and the release of circulating vasoconstrictor hormones such as angiotensin II and antidiuretic hormone (ADH). These responses act together as powerful feedback to compensate blood pressure via increasing total peripheral resistance (TPR).

Severe hemorrhage leads to a higher risk of mortality and morbidity in obese patients (Nelson et al., 2012). However the mechanisms responsible for the increased risks are unclear. Impaired baroreflex control of sympathetic activity and heart rate in obesity (Davis, 2011; Huber and Schreihofer, 2010; Schreihofer et al., 2007) may blunt TPR and heart rate responses following hemorrhage. Also, an elevated basal sympathetic activity in obese individuals and animals (Eikelis et al., 2003; Kalupahana and Moustaid-Moussa, 2012; Mark et al., 1999; Narkiewicz et al., 1998) may limit their ability to increase cardiovascular responses after hemorrhage (Frisbee, 2006). There are virtually no studies that investigate the cardiovascular and hormonal responses to severe hemorrhage in obese subjects or animals under conscious conditions.

Determining blood pressure recovery under conscious conditions is important since anesthesia may interfere with neural and possibly other hormonal responses. Frisbee et al. showed that unconscious obese Zucker rats (OZ) exhibited significantly impaired blood pressure recovery after losing 10% of volume in 4 successive increments (Frisbee, 2006). However, in our previous study, conscious OZ with a 20% loss of volume and an additional 10% loss after a 40-minute recovery did not exhibit impaired blood pressure compensation as compared to lean Zucker rats (LZ) (Xiang et al., 2012). This inconsistency raises the possibility that anesthesia interferes with the blood pressure regulation following hemorrhage differently between lean and obese rats. Additionally, there is evidence that physiologic response to hemorrhagic shock depends on rate and means of hemorrhage (Frankel et al., 2007). Therefore, the current study was designed to determine the regulation of cardiovascular hemodynamics following a single severe hemorrhage (a loss 35% of blood volume) in conscious OZ.

We hypothesized that the blood pressure compensation following severe hemorrhage is impaired in conscious OZ. OZ has been widely used as a model for central obesity with metabolic and autonomic disorders, along with cardiovascular dysfunction, similar to those conditions

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seen in obese subjects. In the current study, we compared hemodynamic parameters, blood gasses, and hormonal responses (ADH and the renin–angiotensin system) between conscious lean Zucker rats (LZ) and OZ before and after severe hemorrhage. This data would provide important insight into the mechanism(s) and the treatment strategies in obese patients with hemorrhagic shock.

Materials and methods

Animals and surgical preparation

Male Zucker rats (11–13 weeks), Harlan Laboratories, had mean bodyweights of 306 ± 7 g for LZ and 452 ± 10 g for OZ. The experimental protocols for this study were approved by the Institutional Animal Care and Use Committee at the University of Mississippi Medical Center and were carried out according to both the "Guide for the Care and Use of Laboratory Animals" from the National Institutes of Health and also the guidelines of the Animal Welfare Act. All the rats were housed 2–3 animals per cage at 22 °C (12-h light–dark cycle) with food and water ad libitum.

Animals were anesthetized with inhalation of ~4–6% of isoflurane combined with 100% O_2. The neck and left hindlimb were shaved lightly and wiped with 70% ethanol. The right jugular, left carotid artery, and left femoral artery were isolated, catheterized (catheters containing 10% heparin in saline), and then exteriorized. Incisions were closed using 4-0 vicryl. After recovery from anesthesia, blood pressure and heart rate were recorded via carotid catheter (PowerLab system, Model: ML 118). Before each set of experiments, animals were allowed to equilibrate from surgery for 2–3 h until the blood pressure and heart rate reached steady-state levels.

Baroreflex sensitivity and basal sympathetic tone

To measure baroreflex sensitivity, blood pressure and heart rate were recorded before and after administration of a bolus of the vasodilator sodium nitroprusside via jugular catheter. Three different doses of sodium nitroprusside (10, 50, and 100 μg/kg) were used for LZ. The doses used for OZ were estimated by using the mean bodyweight of the LZ, because the blood volumes of age-matched LZ and OZ are similar (Frisbee, 2006; Schreihofer et al., 2005). The volume of sodium nitroprusside (SNP) for each bolus was less than 0.15 ml, so as to have minimal impact on blood volume. The SNP-induced decrease in blood pressure only lasted for 3–5 min. The length of time of the decreased blood pressure and the time point of the peak changes in blood pressure were variable between animals. Therefore, we compared the baroreflex control of heart rate when the maximal presser effect was achieved, similar to a previous report (Schreihofer et al., 2007). The rats were allowed to equilibrate 15 min or until the blood pressure and heart rate reached steady-state levels between each injection.

In an additional set of experiments, the basal adrenergic vascular tone was assessed by measuring blood pressure in LZ and OZ before and 5 min after administration of a bolus of prazosin (α_1-receptor antagonist, 1 mg/kg) via jugular catheter to estimate the basal adrenergic tone in LZ and OZ.

Hemodynamics before and after hemorrhage in LZ and OZ

Rats were placed in a Columbus Instruments metabolic cage to monitor oxygen consumption. Rats had no access to food or water during the experiment. Arterial and venous blood samples (<0.2 ml each) were collected from femoral and jugular catheters, respectively, to measure the basal levels of blood gasses (Trupoint Blood Analysis System). This minor loss of blood volume did not affect blood pressure and heart rate.

After another 30-minute equilibration in the metabolic cage, hemorrhage was induced by withdrawing 35% of total blood volume from the femoral catheter (~0.5 ml/min, 6–8 ml). The total blood volume in LZ was estimated using the formula: body weight × 0.06 + 0.77 (Boku et al., 2010; Lee and Blaufox, 1985). The total blood volume in OZ was estimated by using the mean bodyweight of the LZ since there is evidence that the total blood volume is not different between age-matched LZ and OZ, despite the difference in body weight (Frisbee, 2006; Schreihofer et al., 2005).

Because our goal was to measure the acute responses to hemorrhage in LZ and OZ, we chose to measure the blood pressure and heart rate both during and for 1 h following hemorrhagic blood withdrawal. The first milliliter of arterial blood from the hemorrhage was collected for measurements of arterial blood gasses, plasma renin activity (PRA) and ADH levels (radioimmunoassay, Diagnostic Products). The subsequent blood drawn was used to measure hematocrit by the ratio of the length of the column of blood cells relative to the length of the column containing the entire sample after being centrifuged at 3000 *g* for 15 min.

After 1 h of recovery, blood samples were collected again to measure blood gasses, hematocrit, PRA, and ADH levels. To minimize blood loss or any other unexpected stress, we did not take blood samples during the recovery period. Using blood gas and hematocrit measurements, the arterial and venous oxygen contents (O_2 ml/ml) were derived using the following formula: hematocrit × 0.34 × 100 × 1.36 × oxygen saturation + 0.0031 × oxygen partial pressure. Based on the Fick principal, cardiac output (ml/min) = oxygen consumption × bodyweight × 100 / (arterial oxygen content − venous oxygen content). TPR (mm Hg·min/ml) = mean arterial pressure / cardiac output.

Quantitative and statistical analyses

Data were compared by using two-way repeated measures ANOVA. Where significant effects occurred, individual groups were compared using the Holm–Sidak method. All the data are presented as means ± SE. A probability of $P < 0.05$ was accepted as statistically significant for all comparisons.

Results

Baroreflex sensitivity and basal sympathetic tone

Fig. 1A and B represent the lowest blood pressure and corresponding heart rate, respectively, after each intravenous injection of SNP. The basal blood pressure and heart rate were not significantly different between LZ and OZ. Administration of SNP caused a similar decrease in blood pressure in both LZ and OZ in a dose-dependent manner (Fig. 1A). However, the increase in heart rate in response to each dose of SNP was significantly blunted in OZ as compared to LZ (Fig. 1B). The blood pressure and heart rate returned to their baseline within 5 min after SNP injection.

In a separate group of animals, prazosin injection resulted in an immediate decrease in blood pressure and increase in heart rate, which reached a steady level within 5 min. Therefore, the blood pressure and heart rate 5 min after the treatment were compared between groups. OZ exhibited a greater decrease in the basal blood pressure as compared with LZ (Fig. 1C). The increases in heart rate after the treatment were significantly blunted in OZ as compared with LZ (Fig. 1D).

Blood pressure and heart rate responses to hemorrhage

The basal blood pressure was not significantly different between LZ and OZ (Fig. 2A). Hemorrhage significantly decreased the blood pressure in all animal groups. The blood pressure was not different between LZ and OZ immediately after or 5 min after hemorrhage (Fig. 2). However, at 30 and 60 min of recovery, OZ exhibited a significantly lower blood pressure as compared with LZ.