Impaired blood pressure recovery to hemorrhage in obese Zucker rats with orthopedic trauma

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Xiang L, Lu S, Fuller W, Aneja A, Russell GV, Jones LB, Hester R. Impaired blood pressure recovery to hemorrhage in obese Zucker rats with orthopedic trauma. Am J Physiol Heart Circ Physiol 302: H340–H348, 2012. First published October 14, 2011; doi:10.1152/ajpheart.00439.2011.—We have shown that obese Zucker rats with orthopedic trauma (OZT) exhibit a loss of arteriolar tone in skeletal muscle. We hypothesize that the loss of arteriolar tone in OZT blunts vasoconstrictor responses to hemorrhage, resulting in an impaired blood pressure recovery. Orthopedic trauma was induced with soft tissue injury and local injection of bone components in both hindlimbs in lean (LZT) and OZT (11–13 wk). One day after the orthopedic trauma, blood pressure responses following hemorrhage were measured in conscious control lean, control obese, LZT, and OZT. In another set of experiments, the spinoتراzezius muscle of control and trauma animals was prepared for microcirculatory observation. Arteriolar responses to phenylephrine (PE) or hemorrhage were determined. Hemorrhage resulted in similar blood pressure responses in control animals and LZT, but the blood pressure recovery following hemorrhage was blunted in the OZT. In the spinoتراzezius, OZT exhibited decreased arteriolar tone and blunted vasoconstrictor responses to PE and hemorrhage. Treatment with glibenclamide improved the blood pressure recovery in the conscious OZT and improved the arteriolar tone, and PE induced vasoconstriction in the spinoتراzezius of the OZT. Thus, ATP-dependent K+ channel-mediated loss of arteriolar tone in OZT blunts the arteriolar constriction to hemorrhage, resulting in impaired blood pressure recovery.

Impaired blood pressure recovery to hemorrhage in obes

ORTHOPEDIC TRAUMA IS THE SECOND most common cause of preventable disability, with ~100,000 deaths annually (3, 7). Severe injuries and/or posttrauma surgeries may increase the risk of hemorrhage (traumatic shock), resulting in hemodynamic instabilities, multiple organ failure, and exaggerated inflammatory responses. Increasing evidence shows that obese patients with severe orthopedic trauma and hemorrhagic shock exhibit a higher risk of mortality and morbidity compared with the nonobese patients (7, 8). However, the mechanisms responsible for the exaggerated impacts of traumatic shock in obesity are unknown.

During early stages of hemorrhagic shock, peripheral vascular resistance is increased via an elevated sympathetic tone to minimize the fall in blood pressure. The peripheral vasoconstrictor responses in skeletal muscle, skin, or the spleen can shunt blood supply to vital organs such as the brain and heart. However, with greater than a 30% loss of effective blood volume (grade 3 and 4), the compensatory mechanisms begin to fail and profound hypotension will develop and can lead to end-organ damage and death (42). This decomposition is due in part to low tissue perfusion and resultant hypoxia and vasodilator metabolite accumulation, which can blunt the sympathetic vasoconstriction, known as “sympathetic escape.”

Obese Zucker rats (OZ) have been widely used as a model of obesity and metabolic syndrome. Our published studies have demonstrated that, compared with lean controls, OZ at 11–13 wk of age exhibit severe obesity and insulin resistance with no hypertension [mean arterial pressure (MAP)] (45, 49). In addition, we have shown that the α1-adrenergic vasoconstriction is similar between 11- to 12-wk-old lean Zucker rats (LZ) and OZ (28). Therefore, to minimize differences in basal sympathetic tone, 11- to 13-wk-old LZ and OZ were chosen for the current study. We previously demonstrated a decreased skeletal muscle arteriolar tone in the OZ with orthopedic trauma (OZT), with the decreased arteriolar tone normalized following indomethacin administration (46). These results suggest that orthopedic trauma leads to a loss of arterial tone in OZT because of increased circulating levels of vasodilator prostaglandin(s). For example, we found increased plasma prostaglandin E2 (PGE2) levels in the OZT but not in the LZ following orthopedic trauma (LZT) (46). This increased vasodilator prostaglandin(s) in OZT may override the vasoconstrictor effect mediated by sympathetic activation and blunt the blood pressure compensation to a mild hemorrhage. Because the vasodilator prostaglandins such as PGE2 and prostacyclin act through the ATP-dependent K+ (KATP) channel (1, 48), the current study focused on effects of basal KATP channel activation on the loss of arteriolar tone, vasoconstrictor response, and blood pressure recovery during hemorrhage in OZT. We hypothesized that a KATP channel-dependent loss of arteriolar tone in OZT blunts vasoconstrictor responses to hemorrhage, resulting in impaired blood pressure recovery. To test this hypothesis, we performed a moderate hemorrhage (grade 2, 20 and 30% loss of total volume) in both nontrauma and trauma lean and obese rats and determined the blood pressure and heart rate responses in conscious animals before and after treatment with the KATP channel inhibitor glibenclamide. We also determined arteriolar responses in the spinoتراzezius muscle following the hemorrhage.

METHODS

Animals. Male LZ and OZ (11–13 wk) were acquired from Harlan Laboratories; mean body weights were 327 ± 8 g for LZ and 503 ± 18 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

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the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health and the guidelines of the Animal Welfare Act. All rats were housed two to three animals per cage at 22°C (12:12-h light-dark cycle) with free access to food and water.

Animal model of orthopedic trauma. Approximately 90% of patients with severe trauma suffer long bone fractures along with an associated soft-tissue injury (9). Therefore, we mimicked a bilateral femur fracture in male LZ and OZ by soft tissue injury followed by the sterile injection of a bone component suspension into the injured thigh muscle as previously described (29, 46). In brief, 11- to 13-week-old LZ and OZ (bone donors) were anesthetized using pentobarbital (50 mg/kg ip), and both femur and tibia bones were harvested under sterile conditions. The bone along with marrow was crushed with a mortar and pestle and homogenized in PBS [3.2 mM Na2HPO4, 0.5 mM KH2PO4, 1.3 mM KCl, and 135 mM NaCl, pH 7.4] (2 g/5 ml). Animals (bone donors) were killed with an overdose of pentobarbital after the collection of bone components. The LZ and OZ (bone recipients, 11–13 wk) were anesthetized and subjected to a soft tissue injury on both hindlimbs by crushing the middle section of the muscle group behind the femur with a Kelly clamp (51/2 in., 14 cm) at the first notch for 30 s, followed by the sterile injection of the bone component suspension (1.5 ml/kg) into the injured muscles using a 18-gauge needle, lean to lean and obese to obese. We applied lean fragments to lean animals and obese fragments to obese animals to minimize any unexpected immune responses. Our published study has demonstrated increased circulating PGE2 and cytokine levels along with pulmonary injury in the OZT induced by this protocol (46). This trauma model does not need posttrauma treatments such as surgery or antibiotics, which allows us to focus on the impact of orthopedic trauma. The following animals were used: LZ, OZ, LZT, and OZT.

Experiments were performed on the first day after orthopedic trauma.

Blood pressure recovery following hemorrhage in conscious animals. One day following orthopedic trauma, rats (LZ, OZ, LZT, and OZT) were anesthetized with isoflurane inhalation, and a catheter with 10% heparin was implanted in the right carotid artery. After recovery from anesthesia, the rats were allowed to equilibrate for 6 h, and the baseline blood pressure and the blood pressure responses to moderate grade 2 hemorrhage, 20% followed by an additional 10% loss of total blood volume 40 min later, were measured. Hemorrhage was induced by spontaneous bleeding from the carotid catheter (3 ± 3 ml/min). In a separate experiment, the OZT was injected with the KATP channel inhibitor glibenclamide (5 mg/kg ip) (46, 47), and hemorrhage was induced ~80 min after the injection. The blood pressure and heart rates were recorded before and every 5 min for 40 min following each hemorrhage (model: ML 118, PowerLab). The measurement of blood pressure and heart rate in the conscious animals excludes the effect of anesthesia on sympathetic activity. During the experiment, the rats were kept in a covered cage with no access to water. The total blood volume in LZs was estimated using “body wt × 0.06 + 0.77” (25). The total blood volume in OZs was estimated by using the mean body weight of the LZ, since there is evidence that the total blood volume is not different between LZs and OZs despite the difference in body weight (13, 36). The blood from the first hemorrhage was centrifuged at 3,100 g for 15 min, and hematocrit was calculated by the ratio of the length of the column of blood cells relative to the length of the column of the whole specimen.

Basal arteriolar tone following orthopedic trauma. One day after orthopedic trauma, LZ, OZ, LZT, and OZT were anesthetized with pentobarbital (50 mg/kg ip), and the right spinotrapezius muscle was prepared for the experimental observation as previously described (15, 47). The left jugular vein was cannulated for supplemental addition of anesthesia. The trachea was intubated for the animals to spontaneously breathe a gas mixture containing 30% oxygen and 70% nitrogen. The body core temperature was maintained from ~37 to 38°C with a heating lamp. During surgery and subsequent experiments, the spinotrapezius muscle was kept at in situ dimensions and continuously superfused with a PSS aerated with a 5% CO2–95% N2 gas mixture (pH = 7.4, 35°C) to ensure the oxygen was mainly supplied by the blood. Superfusate flow was maintained at 4–6 ml/min to minimize equilibration with atmospheric O2. The microcirculation of the spinotrapezius muscle was transilluminated and observed with a Nikon microscope fitted with a ×10 water immersion objective (numerical aperture = 0.30). The microscopic image was televised with a Dage closed-circuit television camera and displayed on a Sony monitor. The magnification of the image was ×660 from the tissue to the monitor screen. Vessel diameter was measured by using a Texas A&M video analyzer modified to function as a video micrometer. The resolution of this system was ± 1 μm.

Animals were allowed to stabilize for 15–30 min after completion of the surgical procedure. A segment of arcade arteriole with clear luminal diameter was randomly chosen to study. The basal arteriolar diameters (bas) were measured before and after administration of the KATP inhibitor glibenclamide through the superfusion solution to yield a final concentration of 10−6 M for 30 min in LZT and OZT. At the end of each experiment, 10−4 M of adenosine (ADO) plus 10−6 M of sodium nitroprusside (SNP) were used to achieve the maximal diameter (MAX). Arteriolar tone was calculated by (MAX − BAS)/MAX × 100%. Therefore, a vasodilator response would result in a decrease in vascular tone.

Blood pressure recovery and vasconstrictor responses in unconscious animals. One day after orthopedic trauma, LZ, OZ, LZT, and OZT were anesthetized with pentobarbital, and the right spinotrapezius muscle was prepared for the experimental observation. The left jugular vein was cannulated for supplemental addition of anesthetic. After 30 min of equilibration, the vasconstrictor responses to increasing concentrations of phenylephrine (PE, 10−6, 10−5, 10−4, and 10−3 M) in the superfusion solution were measured. After the PE was washed out and the arteriolar diameters returned to baseline, the vessels in LZT and OZT were treated with glibenclamide (10−6 M) for 30 min, and the PE (10−6, 10−5, 10−4, and 10−3 M)-induced vascular responses were measured.

In another set of experiments, the blood pressure and arteriolar diameter in the spinotrapezius muscle in response to hemorrhage were observed. After 30 min of equilibration, the blood pressure and vasconstrictor responses to 20 or 30% (additional 10%) loss of the total blood volume were measured. Hemorrhage was induced by spontaneous bleeding from the carotid catheter (~3 ml/min). The blood pressure and arteriolar diameters were recorded before and every 5 min for 40 min following each hemorrhage (DataQ). In an additional group of animals, the OZT was injected with the KATP channel inhibitor glibenclamide (5 mg/kg ip). To validate the inhibitory effects of glibenclamide, the basal arteriolar diameter and cromakalim-induced vasodilation were measured ~80 min after the injection. Cromakalim was given through the superfusion solution to yield a final concentration of 10−6 M. After cromakalim was washed out and the arteriolar diameter returned to the basal level in the presence of glibenclamide, the vasocconstrictor responses to 20 or 30% (additional 10%) loss of the total blood volume were measured.

PGE2-induced vasodilation in nontrauma rats. Nontrauma LZ and OZ were anesthetized, and the right spinotrapezius muscle was prepared for experimental observation. After 30 min of equilibration, the vasodilator responses to PGE2 (via superfusion solution, 10−7 and 10−5 M) were determined before and after treatment with glibenclamide (via superfusion solution, 10−6 M, for 30 min). At the end of each experiment, ADO plus SNP was added in the superfusion solution to determine the maximal diameter.

PE-induced vasconstriction in PGE2-pretreated nontrauma animals. LZ and OZ were anesthetized, and the right spinotrapezius muscle was prepared for experimental observation. The vascular responses to increasing concentrations of PE (10−9, 10−8, 10−7, and 10−6 M) in the superfusion solution were measured. After the PE was washed out and the arteriolar diameters returned to the baseline, the vessels were pretreated with PGE2 (5 × 10−6 M) via the superfusion solution. The PGE2 concentration was chosen to induce a similar loss of arteriolar tone.
tone in LZ and OZ compared with OZT. After stable arteriolar diameters were achieved, the vascular responses to increasing concentrations of PE (10⁻⁸, 10⁻⁷, 10⁻⁸, and 10⁻⁶ M) were repeated.

Data analysis and statistical methods. Data were collected in a personal computer. The dose responses to PGE2 and PE were analyzed using two-way repeated-measures ANOVA. The blood pressure, heart rates, and arteriolar diameters in response to hemorrhagic shock within obese rats with or without glibenclamide treatment were analyzed using a one-way ANOVA. The blood pressure, heart rates, and arteriolar diameters in response to hemorrhagic shock were compared with their basal levels by using one-way repeated-measures ANOVA within each group. The other data were compared between groups using two-way ANOVA. Where significant effects occurred, individual groups were compared using the Holm-Sidak method. All of the other data are presented as means ± SE. A probability of P < 0.05 was accepted as statistically significant for all comparisons.

RESULTS

Heart rates and blood pressure recovery following moderate hemorrhage in conscious animals. Figure 1 shows that hematocrits were not different among LZ, OZ, LZT, and OZT. In addition, glibenclamide treatment (5 mg/kg ip) in OZT did not alter the hematocrit (data not shown). The heart rates and blood pressure in response to hemorrhage in conscious animals are shown in Figs. 2 and 3, respectively. The basal heart rates and MAP were not different among groups.

The heart rate responses following hemorrhage in conscious LZ, OZ, LZT, and OZT are presented in Fig. 2A. In the LZ, the heart rates after losing 20% or 30% of total blood volume were not altered compared with their basal levels. There was only a transient increase in the heart rate immediately after 30% of hemorrhage (at 0 min) in LZ. In the LZT, the heart rates were increased immediately after (at the 0 min) loss of 20 or 30% of total blood volume. In addition, the heart rates in LZT were higher compared with the basal levels and the other groups at the first 25 min after losing 20% of total blood volume. Heart rates were not different between OZ and OZT. However, the OZ and OZT rats exhibited lower heart rates than the LZ or LZT from 30 to 40 min after losing 20% of total blood volume and throughout the whole 40-min recovery period after losing 30% of total blood volume. In addition, the heart rates in OZ and OZT were decreased compared with their basal levels from 5 to 15 min after losing 20% of total blood volume and from 5 to 40 min after losing 30% of total blood volume. The data of OZ and OZT in Fig. 2B are the same data presented in Fig. 2A.

In Fig. 2B, hemorrhage resulted in a similar decrease in blood pressure in conscious LZ, OZ, LZT, and OZT followed by gradual increases in pressure during the 40-min recovery period. After losing 20% of total blood volume, the blood pressure was restored by the 25th min in both LZ and LZT. However, the blood pressures were not restored in OZ and OZT throughout the 40-min recovery period compared with their basal levels. Additionally, the OZT exhibited a lower MAP from 25 to 40 min compared with the other groups. After losing 30% (additional 10%) of total blood volume, the blood pressures in the LZ, OZ, and LZT were partially restored by 40 min and were not significantly different from each other. However, the MAP in OZT from 25 to 40 min was significantly lower compared with the other groups.

Figure 3A compares the heart rate responses in OZ, OZT, and OZT treated with glibenclamide. Neither orthopedic trauma nor glibenclamide altered the basal heart rates in obese rats or the heart rates following hemorrhage. The MAP in OZ, OZT, and OZT treated with glibenclamide are presented in Fig. 3B. Glibenclamide treatment had no effect on the basal MAP but improved MAP recovery in the OZT after losing 20 or 30% of total blood volume. The OZ and OZT data in Fig. 3 are the same data presented in Fig. 2.

Basal arteriolar tone. The basal and maximal arteriolar diameters in LZ, OZ, LZT, and OZT the first day following orthopedic trauma are presented in Table 1. The basal diameters were not different between the LZ and OZ. Orthopedic
trauma significantly increased the basal arteriolar diameter in the obese rats with no effect in the lean animals. Treatment with glibenclamide (via superfusion solution) had no effect on the basal arteriolar diameters in LZ, OZ, or LZT but decreased the basal arteriolar diameter in OZT to the levels observed in the other groups. The maximal diameters induced by ADO and SNP were not different between the nontrauma animals and LZT but were significantly larger in OZT. Losing 20 or 30% (additional 10%) of total blood volume, the blood pressure was restored by the 40-min recovery period in the nontrauma animals and LZT. In the LZ and LZT, the arteriolar diameters were smaller than in OZ, OZT, and OZT treated with glibenclamide. The OZ and OZT data are the same as in A. Glibenclamide treatment had no effect on the basal MAP. The blood pressure is not different between OZ and OZ + glibenclamide (*P < 0.05, OZT vs. the other groups; #P < 0.05, OZ + glibenclamide vs. OZT; n = 11 for LZ and OZ; n = 12 for LZT and OZT).

Blood pressure recovery and vasoconstrictor responses in anesthetized animals. Figure 4 shows the blood pressure recovery in anesthetized LZ, OZ, LZT, and OZT. The basal blood pressure is higher in the OZ than in the LZ. Hemorrhage resulted in a similar decrease in blood pressure in conscious LZ, OZ, LZT, and OZT followed by a gradual increase in pressure during the 40-min recovery period. After losing 20% of total blood volume, the blood pressure was restored by the 30th min in LZ and LZT. However, the blood pressures were not restored in the OZ until the 40th min after the hemorrhage, whereas the OZ exhibited higher blood pressure from 35 to 40 min compared with the other groups. The blood pressure in OZT failed to recover throughout the 40-min recovery period. Additionally, the OZT exhibited a lower MAP from 25 to 40 min compared with the other groups. After losing 30% (an additional 10%) of total blood volume, the blood pressures in the LZ, OZ, and LZT were restored partially by 40 min, whereas the OZ exhibited higher blood pressure from 30 to 40 min compared with the other groups. However, the MAP in OZT from 15 to 40 min was significantly lower compared with the other groups.

The arteriolar responses to hemorrhage in LZ, OZ, LZT, and OT are shown in Fig. 5A. The basal arteriolar diameters were not different between the nontrauma animals and LZT but were significantly larger in OZT. Losing 20 or 30% (additional 10%) of total blood resulted in an immediate increase in arteriolar diameters followed by gradual vasoconstrictor response during the 40-min recovery period in the nontrauma animals and LZT. In the LZ and LZT, the arteriolar diameters were smaller than their basal levels from 30 to 40 min after losing 20% of total blood volume and from 35 to 40 min after losing 30% of total blood volume. In the OZ, the arteriolar diameters were smaller than its basal levels from 30 to 40 min after losing 20% of total volume compared with the other groups.

Table 1. The effect of orthopedic trauma on the arteriolar tone

<table>
<thead>
<tr>
<th>Diameter</th>
<th>LZ</th>
<th>OZ</th>
<th>LZ + Glibenclamide</th>
<th>OZT</th>
<th>OZ + Glibenclamide</th>
<th>LZ + PGE2</th>
<th>OZ + PGE2</th>
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<tbody>
<tr>
<td>Basal (BAS), μm</td>
<td>15 ± 1</td>
<td>15 ± 1</td>
<td>17 ± 1</td>
<td>16 ± 1</td>
<td>21 ± 1*</td>
<td>16 ± 1</td>
<td>25 ± 1</td>
</tr>
<tr>
<td>Maximal (MAX), μm</td>
<td>39 ± 3</td>
<td>37 ± 2</td>
<td>43 ± 2</td>
<td>43 ± 2</td>
<td>36 ± 1</td>
<td>39 ± 3</td>
<td>35 ± 1</td>
</tr>
<tr>
<td>Tone (MAX - BAS/MAX), %</td>
<td>59 ± 3</td>
<td>59 ± 2</td>
<td>60 ± 2</td>
<td>62 ± 1</td>
<td>43 ± 2*</td>
<td>59 ± 1</td>
<td>35 ± 3*</td>
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Values are means ± SE; n = 12 for lean Zucker rats (LZ), n = 10 for obese Zucker rats (OZ), n = 11 for LZ with orthopedic trauma (LZT), n = 14 for with orthopedic trauma (OZT), and n = 5 for LZ + prostaglandin E2 (PGE2) and OZ + PGE2. BAS, basal arterial diameter; MAX, maximal diameter. The basal arteriolar diameters were not different among LZ, OZ, and LZT. The maximal diameters induced by sodium nitroprusside and adenosine were not different among LZ, OZ, LZT, and OZT. OZT exhibited larger basal arteriolar diameters and loss of arteriolar tone compared with the other groups. Treatment with glibenclamide decreased the basal diameters and improved the arteriolar tone in OZT with no effect in the other groups. Pretreatment with PGE2 (5 × 10⁻⁶ M) results a similar loss of tone in both LZ and OZ, which is not different from the decreased arteriolar tone observed in OZT. *P < 0.05 vs. the other groups.
blood volume and from 20 to 40 min after losing 30% of total blood volume. OZT exhibited larger arteriolar diameters than LZ, OZ, and LZT before and after hemorrhage. In the OZT, the vasoconstrictor response to the hemorrhage was absent after losing 20 and 30% of total blood volume.

Figure 5B shows that the glibenclamide treatment (5 mg/kg ip) decreased the basal arteriolar diameter and blocked the cromakalim-induced vasodilation in the OZT. Figure 5C shows that glibenclamide treatment (5 mg/kg ip) decreased the basal arteriolar diameters and the arteriolar diameters in response to hemorrhage in OZT. The OZ and OZT data in Fig. 5C are the same data presented in Fig. 5A. The arteriolar diameters of OZT treated with glibenclamide were still larger than the arteriolar diameter of OZ at 30 and 40 min after losing 20% of total blood volume and from 10 to 40 min after losing 30% of total blood volume.

Figure 6A compares the vasoconstrictor responses to PE in the arcade arterioles in spinotrapezius muscles. PE induced similar concentration-dependent vasoconstrictor responses in LZ, OZ, and LZT. However, compared with the other groups, the vasoconstrictor responses to PE were attenuated in the OZT at all concentrations. Only $10^{-6}$ M of PE resulted in significant vasoconstrictor response in the OZT. Figure 6B shows the vasoconstrictor response to PE before and after treatment with glibenclamide in only LZT and OZT. PE induced vasoconstrictor responses in LZT in a concentration-dependent manner, with the vasoconstrictor responses attenuated in the OZT. Treatment with glibenclamide had no effect on the PE-induced vasoconstriction in LZT but restored the vasoconstrictor response in OZT to the same levels as seen in LZT.

Role of KATP channel activation on vasoconstrictor response. The vasodilator responses to PGE2 before and after treatment with glibenclamide in LZ and OZ are presented in Fig. 7A. In this set of experiments, the basal and maximal diameters were not different between the LZ and OZ (BAS = 15 ± 1 in both LZ and OZ; MAX = 33 ± 2 in LZ and 34 ± 1 in OZ). The vasodilator responses to PGE2 were presented as a percentage of the maximal vasodilation. Administration of PGE2 ($10^{-9}$ and $10^{-8}$ M) resulted in a concentration-dependent arteriolar

Fig. 5. A: arteriolar diameters in response to different stages of hemorrhage (*$P < 0.01$, OZT vs. the other groups; #$P < 0.05$ vs. basal within LZ and LZT; &$P < 0.05$ vs. basal within OZ; $\ddot{P} < 0.01$ vs. basal within OZT; $n$ = 5 for each group). B: cromakalim-induced vasodilation before and after glibenclamide (ip) in OZT (*$P < 0.05$, before vs. after within basal; $\ddot{P} < 0.05$ vs. basal or cromakalim after glibenclamide; $n$ = 5). C: glibenclamide treatment (5 mg/kg ip) decreased the basal arteriolar diameters and the arteriolar diameters in response to hemorrhage in OZT. The OZ and OZT data are the same as in A (*$P < 0.01$, OZT vs. the other groups; $\ddot{P} < 0.05$, OZT vs. OZT + glibenclamide; &$P < 0.05$ vs. basal within OZ; $\dddot{P} < 0.01$ vs. basal within OZT; $\dddot{P} < 0.05$ vs. basal within OZT + glibenclamide; $n$ = 5 for each group).

blood volume and from 20 to 40 min after losing 30% of total blood volume. OZT exhibited larger arteriolar diameters than LZ, OZ, and LZT before and after hemorrhage. In the OZT, the vasoconstrictor response to the hemorrhage was absent after losing 20 and 30% of total blood volume.

Figure 5B shows that the glibenclamide treatment (5 mg/kg ip) decreased the basal arteriolar diameter and blocked the cromakalim-induced vasodilation in the OZT. Figure 5C shows that glibenclamide treatment (5 mg/kg ip) decreased the basal arteriolar diameters and the arteriolar diameters in response to hemorrhage in OZT. The OZ and OZT data in Fig. 5C are the same data presented in Fig. 5A. The arteriolar diameters of OZT treated with glibenclamide were still larger than the arteriolar diameter of OZ at 30 and 40 min after losing 20% of total blood volume and from 10 to 40 min after losing 30% of total blood volume.

Figure 6A compares the vasoconstrictor responses to PE in the arcade arterioles in spinotrapezius muscles. PE induced similar concentration-dependent vasoconstrictor responses in LZ, OZ, and LZT. However, compared with the other groups, the vasoconstrictor responses to PE were attenuated in the OZT at all concentrations. Only $10^{-6}$ M of PE resulted in significant vasoconstrictor response in the OZT. Figure 6B shows the vasoconstrictor response to PE before and after treatment with glibenclamide in only LZT and OZT. PE induced vasoconstrictor responses in LZT in a concentration-dependent manner, with the vasoconstrictor responses attenuated in the OZT. Treatment with glibenclamide had no effect on the PE-induced vasoconstriction in LZT but restored the vasoconstrictor response in OZT to the same levels as seen in LZT.

Role of KATP channel activation on vasoconstrictor response. The vasodilator responses to PGE2 before and after treatment with glibenclamide in LZ and OZ are presented in Fig. 7A. In this set of experiments, the basal and maximal diameters were not different between the LZ and OZ (BAS = 15 ± 1 in both LZ and OZ; MAX = 33 ± 2 in LZ and 34 ± 1 in OZ). The vasodilator responses to PGE2 were presented as a percentage of the maximal vasodilation. Administration of PGE2 ($10^{-9}$ and $10^{-8}$ M) resulted in a concentration-dependent arteriolar

Fig. 6. A: vasoconstrictor response to phenylephrine (PE) in the arcade arterioles in spinotrapezius muscles of LZ, OZ, LZT, and OZT. The vasoconstrictor responses were attenuated in the OZT at all concentrations compared with the other groups (*$P < 0.01$, OZT vs. other groups; $n$ = 5 for the LZ and OZ; $n$ = 4 for LZT and $n$ = 6 for OZT). B: vasoconstrictor response to PE before and after treatment with glibenclamide in LZT and OZT. Treatment with glibenclamide restored the vasoconstrictor response in OZT with no effect in LZT (*$P < 0.05$, OZT vs. the other groups; $n$ = 4 for LZT and $n$ = 6 for OZT).
vasodilation in both LZ and OZ. The vasodilator response to 10^{-9} M PGE_2 was attenuated significantly in the OZ compared with the LZ. The difference was not significant after application of 10^{-8} M of PGE_2. Treatment with glibenclamide had no effect on basal arteriolar diameters but significantly inhibited the PGE_2-induced vasodilation in both LZ and OZ. Figure 7B presents PE-induced similar concentration-dependent vasoconstrictor responses in LZ and OZ. Pretreatment with PGE_2 (5 × 10^{-9} M) in both LZ and OZ resulted in a similar loss of arteriolar tone comparable to the arteriolar tone observed in OZT (Table 1) and blunted vasoconstrictor responses to all concentrations of PE.

**DISCUSSION**

The major findings of this study show that 1) OZT exhibited blunted blood pressure recovery following moderate hemorrhage; 2) OZT exhibited a K_{ATP} channel-dependent decrease in arteriolar tone; 3) the K_{ATP} channel-dependent decrease in arteriolar tone was associated with blunted vasoconstrictor responses to PE and hemorrhage; and 4) K_{ATP} channel inhibitor glibenclamide improves vasoconstrictor responses and the MAP compensation following hemorrhage in OZT.

**Effect of obesity and orthopedic trauma on sympathetic tone.** Obesity is associated with elevated sympathetic activity. OZ at 15–17 wk old exhibits hypertension and elevated peripheral adrenergic vasoconstriction compared with LZ (13, 41). However, the blood pressure is not different between younger LZ and OZ at 11–13 wk of age (35, 49). Moreover, the basal heart rate of OZ at ~12 wk of age is not increased compared with LZ and Sprague Dawley rats (2, 6). We found similar results in the current study that the basal MAP and heart rates were not different between 11- to 13-wk-old LZ and OZ. In addition, the α_1-adrenergic vasoconstriction is greater in 16- to 18-wk-old OZ but similar between 11- to 12-wk-old LZ and OZ (28). Consistent with these findings, the current study shows that the blood pressure and PE-induced vasoconstriction in younger OZ (11–13 wk old) were not different from LZ. Thus, the sympathetic tone is not increased in the younger OZ.

An increased sympathetic activity has been suggested as an index of the intensity of surgical or orthopedic trauma (10, 34). However, in the current study, the basal blood pressure and heart rates were not elevated in either conscious LZT or OZT, suggesting minimal trauma-induced sympathetic activation in this animal model of orthopedic trauma. In addition, the basal blood pressure was not altered in the OZT despite the basal vasodilation (Table 1), similar to the ob/ob mice with normal blood pressure and loss of arterial tone (44). These results are also supported by studies that PGE_2 infusion decreases vascular resistance without altering blood pressure (11, 19). Moreover, the hematocrit was not altered in either LZT or OZT (Fig. 1), suggesting that the total blood volume is not affected by orthopedic trauma.

**Heart rate response to moderate hemorrhage in lean and obese rats with or without orthopedic trauma.** During hemorrhage, decreased blood volume is thought to increase heart rates via both an elevated sympathetic outflow and withdrawal of vagal output. However, we found that this is not the case in the rats during moderate hemorrhage. There was only a transient increase in heart rates in the LZT after losing 20% of total blood volume (Fig. 2A). Although an absence of tachycardia during a moderate or severe hemorrhage has been demonstrated in rats (4, 18, 30, 32, and 43), the mechanism responsible for this decreased heart rate following hemorrhage is unclear. A possible mechanism is that, after a loss of >20% of total blood volume, the myocardial mechanoreceptors may be activated because of critically reduced cardiac blood flow and filling pressure (Bezold-Jarish reflex) (27, 38), resulting in increased vagal outflow and decreased arteriovenous conductance (4, 18, 27, 32, and 50). For example, the tachycardia and sympathetic nervous activity were reversed by cervical vagotomy but not by atropine, suggesting an involvement of vagal afferents (43). Thus, the absence of tachycardia during hemorrhage is considered as a protective mechanism against a further cardiac ischemia due to elevated workload (tachycardia and inotropy). We found that the heart rates in obese rats during hemorrhage were lower compared with their basal levels and with the heart rates in lean rats (Fig. 2A). A similar decrease in heart rates after losing >18% of total blood volume was recently demonstrated in the streptozotocin-treated type 1 diabetic rats due to elevated Bezold-Jarish reflex and decreased baroreflex (4). Although an impaired baroreflex is demonstrated in OZ (6, 17, and 37), whether the decreased heart rate following moderate hemorrhage in OZ is the result of altered baroreflex control and/or an elevated Bezold-Jarish reflex has not been determined.

**The blood pressure response to moderate hemorrhage in lean and obese rats with or without orthopedic trauma.** After a moderate hemorrhage without blood transfusion, individuals with normal physical and cardiovascular function can maintain...
blood pressure via compensatory mechanisms. In the current study, the blood pressures in conscious and unconscious LZ and LZT were restored to their basal levels by 25 min after losing 20% of total blood volume (Figs. 3A and 4). However, the blood pressure failed to return to the basal level following 40 min recovery in the conscious OZ (Fig. 3A). In the unconscious OZ, there was an ~15-min lag of blood pressure recovery after losing 20% of total blood volume compared with the lean rats (Fig. 4). These results suggest an impaired hemorrhage tolerance in OZ, similar to Frisbee’s findings (13). Because the OZ exhibited similar arteriolar constriction compared with the lean rats, the impairment of the hemorrhage tolerance in these obese animals may be because of the decreased cardiac output as described above. In addition, it has been shown that OZ exhibits a lower capillary density and thicker capillary basement membrane than LZ even at 11 wk of age (24). These characteristics may imply a reduced total reabsorption capacity due to increased diffusion distance and decreased capillary surface area (12, 24) following hemorrhage. However, it is known that the hematocrit will not change significantly until several hours after an acute hemorrhage; thus, the compensation from the water reabsorption during the 80-min recovery in the current study might be minimal. After losing 30% of total blood volume, the blood pressures failed to completely return to their basal levels in LZ, LZT, and OZ after a 40-min recovery period but were similar among groups. These results are similar with clinical observations that a grade 3 hemorrhage (losing 30% of total blood volume) results in a decompensated blood pressure.

Hemorrhage following severe injuries and/or posttrauma surgeries in obese subjects result in a higher risk of mortality and morbidity compared with nonobese patients (7, 8). In both conscious and unconscious lean animals, orthopedic trauma did not affect the blood pressure responses to 20 or 30% total hemorrhage. However, the blood pressure recovery following each hemorrhage was impaired in both conscious and anesthetized OZT (Figs. 3 and 4). Moreover, orthopedic trauma in OZT had no effect on the posthemorrhage heart rate but blunted the arteriolar constriction. Glibenclamide treatment partially restored the vasoconstrictor response as well as the blood pressure recovery in OZT without affecting the heart rates. Thus, these results suggest that the impaired blood pressure recovery in OZT is due, at least in part, to a K\textsubscript{ATP} channel-mediated alteration in the vasoconstrictor response.

\textit{K}\textsubscript{ATP} channel-mediated loss of arteriolar tone in OZT. We induced orthopedic trauma in the hindlimbs of OZT and observed a vasodilation in the spinotrapezius muscle, with the arteriolar tone normalized after treatment with the K\textsubscript{ATP} channel inhibitor glibenclamide. These results suggest that the basal vasodilation in OZT is due, at least in part, to elevated circulating K\textsubscript{ATP} channel opener(s). However, it should be realized that trauma-induced inflammatory responses may increase the production of various vasomotor factors. These factors may include histamine, prostacyclin, thromboxane A\textsubscript{2} (50), free oxygen radicals, and inflammatory cytokines, which may alter vascular tone via combined vasodilator and vasoconstrictor effects.

Our previous study showed increased inflammation and circulating PGE\textsubscript{2} levels along with a loss of arteriolar tone in the OZT within three days following orthopedic trauma (46). PGE\textsubscript{2} is a K\textsubscript{ATP} channel-dependent vasodilator (1), and the current study shows that glibenclamide inhibited the majority of the vasodilator response to PGE\textsubscript{2} (Fig. 4). PGE\textsubscript{2} production is associated with elevated inflammation after orthopedic trauma in obesity (3, 14). Elevated inflammatory cytokines can increase the production of PGE\textsubscript{2} within 24 h (21). In addition, the elevated circulating PGE\textsubscript{2} levels and loss of arteriolar tone in OZT were normalized following a systemic treatment with a cyclooxygenase inhibitor (46). Thus, PGE\textsubscript{2} may be (one of) the K\textsubscript{ATP} opener(s) that decreases arteriolar tone in OZT. Whether similar responses would be found in larger vessels or other vascular beds found in regions other than skeletal muscles is unknown. For example, PGE\textsubscript{2} has been reported to cause cerebral and coronary arterial constriction via the activation of EP\textsubscript{1} and EP\textsubscript{3} receptors (20, 26). In addition, PGE\textsubscript{2}-induced vasoconstriction was demonstrated in larger arterioles (~90 \textmu m) in skeletal muscle of \textit{db/db}→+/− mice (a model of type 2 diabetes) due to elevated EP\textsubscript{1} activation (33). The mechanisms and sources for the elevated circulating levels of K\textsubscript{ATP} channel opener(s) in OZT are unknown.

Blunted vasoconstrictor response to hemorrhage in OZT. In response to hemorrhage, peripheral vascular resistance is increased because of elevated sympathetic activity, such as the activation of the baroreflex. However, it should be realized that the feedback of the baroreflex and increased circulating catecholamines to maintain blood pressure are only predominant during a mild hemorrhage, since the threshold of baroreflex is close to 80 mmHg. With a further decrease in the blood pressure, multiple important vasomotor hormones are released to mediate cardiovascular responses and maintain the blood pressure. For example, a posthemorrhage blood pressure around 70 mmHg significantly increases circulating angiotensin II, norepinephrine, endothelin, and vasopressin (AVP) in dogs, resulting in an elevated systemic vascular resistance (16). In the current study, the blood pressure decreased to around 70 mmHg within the first 5 min after losing 20 or 30% of total blood volume. Thus, the vasoconstrictor responses during the recovery period (Fig. 5A) in the LZ, LZT, and OZ could be due to multiple circulating vasomotor hormones. Additionally, the baroreflex has been shown to be impaired in OZ (6, 17, and 37). If this is the case, the blood pressure in OZ may also be maintained by some other means, possibly an elevated vasoconstrictor hormone release.

The arteriolar vasoconstriction without increasing heart rate during hemorrhage in the lean rats and nontrauma obese rats further suggests the production of sympathetic-independent vasoconstrictor hormones (16). In addition, this “inconsistency” between the cardiac and arteriolar responses may be also due to a “regional difference” in autonomic response during hemorrhage. For example, hemorrhage induces opposite neural reflexes in the kidneys and adrenal glands of rats, with vagal tone predominant in the kidneys and sympathoexcitation in the adrenal glands (43). Following hemorrhage, the heart rates in OZT were not different compared with the OZ, suggesting that the blunted vasoconstriction in OZT is not induced by an altered Bezold-Jarisch reflex and sympathetic tone.

The current study showed an impaired PE-induced vasoconstriction in the OZT. Normalizing the arteriolar tone after treatment with glibenclamide improved the PE-induced vasoconstriction (Fig. 6), suggesting that the blunted vasoconstriction in OZT was the result of activated K\textsubscript{ATP} channels rather than decreased adrenergic sensitivity or impaired smooth mus-
cle function. To support this result, we examined PE-induced vasocostriction of PGE2-pretreated arterioles that had an arteriolar tone similar to OZT (Table 1). This allowed us to directly determine the effect of KATP channel-mediated loss of arteriolar tone on the vasocostructor response. As shown in Fig. 7B, the PE-induced vasocostriction was blunted in both LZ and OZ after a pretreatment of PGE2. This was consistent with a previous study showing impaired vasocostructor responses after treatment with PGE2 in rats (11, 40). These results suggest that the arteriolar constriction in OZT is attenuated because of KATP channel-mediated loss of arteriolar tone.

As shown in Fig. 5A, there was an absence of vasocostructor responses in the OZT following 20 or 30% loss of total blood. Based on our findings, the lack of arteriolar constriction in OZT following hemorrhage is due, at least in part, to the KATP channel-mediated loss of arteriolar tone. This is confirmed by the improved hemorrhage-induced vasocostriction following glibenclamide treatment in OZT (Fig. 5C). However, glibenclamide treatment failed to restore the vasocostructor response, raising the possibility of a blunted vasocostructor response to other vasocostructor hormones. Together, an impaired response to vasocostructor hormones may occur early in the OZT even in response to a moderate shock.

Limitations of the current study. The current study found an impaired vasocostructor response in the small arterioles (diameter <30 μm) during hemorrhage in OZT, whereas the response in larger resistance arterioles and small arteries, where 40–55% of the total network resistance resides (31), is unknown. Compared with the small arterioles, larger arterioles may exhibit less vasodilator capability (23) and more profound α1-receptor-mediated vasocostriction (29). In addition, the current study focused on the arteriolar response in skeletal muscle, and whether similar responses occur in other vascular beds after hemorrhage is unknown.

Summary and perspectives. In summary, the blood pressure recovery in OZT following moderate hemorrhage was impaired, at least in part, to a KATP channel-dependent decrease in arteriolar tone, which attenuated the vasocostructor response to hemorrhage. To our knowledge, the current study is the first to demonstrate that, in OZ, a trauma-induced decrease in arteriolar tone leads to an impaired compensatory response to moderate hemorrhage. Future studies are needed to determine the underlying mechanisms responsible for the decreased arteriolar tone in the OZT group after orthopedic trauma and its effect on blood flow distribution in different tissues following hemorrhage.

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DISCLOSURES

We have no conflicts to disclose.

REFERENCES


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