Blood flow, vascular resistance, and blood volume after hemorrhage in conscious adrenalectomized rat

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Darlington, Daniel N., and Majid J. Tehrani. Blood flow, vascular resistance, and blood volume after hemorrhage in conscious adrenalectomized rat. J. Appl. Physiol. 83(5): 1648–1653, 1997.—Hemorrhage leads to cardiovascular collapse and death in adrenal-insufficient animals. To determine whether the cardiovascular collapse is due to vasodilatation and/or failure to restore blood volume, we used radiolabeled microspheres and 125I-labeled albumin to measure blood flow and blood volume in conscious adrenalectomized (ADX) rats after 15 ml·kg\(^{-1}\)·3 min\(^{-1}\) hemorrhage. In ADX rats, hemorrhage led to a greater fall than in sham rats in blood flow in the stomach, small intestines, cecum, colon, spleen, hepatic portal vein, kidney, testis, lung, thymus, bone, fat, forebrain, cerebellum, and brainstem. The greater fall in blood flow was caused by an increase in vascular resistance in these organs except brain and hepatic artery. Sham rats maintained or increased brain and hepatic artery blood flow after hemorrhage whereas flow decreased and remained depressed in ADX rats. ADX rats failed to restore blood volume, whereas sham rats completely restored blood flow by 2 h. We conclude that cardiovascular collapse in ADX rats does not result from vasodilatation but may result from a failure to restore blood volume. The failure to restore blood volume and the low blood flow to organs, especially brain and liver, may contribute to mortality in ADX rats after hemorrhage.

Adrenalectomy

The latter stage of adrenal insufficiency is associated with cardiovascular collapse and heart failure in mammals after various stressors (2, 18) including hemorrhage (3, 8). It has been hypothesized that the cardiovascular collapse is due to the insensitivity of the vasculature to vasoactive agents (1, 2, 11, 18, 19). Although there is considerable evidence demonstrating this phenomenon, we have questioned this hypothesis after showing little change in the vascular sensitivity to vasoactive agents in conscious adrenalectomized (ADX) rats (6, 8). In this model, arterial blood pressure partially recovers after hemorrhage of 15 ml/kg body weight but subsequently falls to very low levels that result in death (3, 7, 8). This is contrary to the responses of conscious adrenalin-intact rats in which both blood volume and blood pressure are restored 2–4 h after large hemorrhage (4, 5). In adrenal-intact rats, the restoration of arterial blood pressure and blood volume is partially due to vasoconstriction of most vascular beds (5); this leads to an elevation in total peripheral resistance and an increase in the movement of fluid from the interstitial space into the vascular space by Starling mechanisms (4, 9, 15, 17). We have shown that conscious ADX rats fail to restore arterial blood pressure after hemorrhage (3, 6–8). However, it is not known whether this results from a failure to increase regional vascular resistance through vasoconstriction and/or from a failure to restore blood volume.

In this study, we measured the changes in blood flow, vascular resistance, and blood volume after hemorrhage in ADX rats. We found that hemorrhage of ADX rats compared with sham-ADX (sham) rats led to a greater vasoconstriction and fall in blood flow in almost all vascular beds studied. However, blood flow to the brain and hepatic artery fell after hemorrhage in ADX rats, whereas blood flow in sham rats remained constant or increased, suggesting autoregulation. Furthermore, unlike sham rats, ADX rats failed to restore blood volume.

Materials and Methods

Animal preparation. Male Sprague-Dawley rats weighing 300–400 g were prepared as described previously (5). All rats were anesthetized with pentobarbital sodium (50 mg/kg ip), and cannulas were inserted into the right carotid and left femoral arteries (Dural Plastics; Auburn, NSW, Australia) to inject 125I-labeled albumin and microspheres, to withdraw blood, and to record arterial blood pressure and heart rate. The cannulas were tunneled under the skin of the back, and they exited through a stainless steel spring that was attached to the skin of the back. The free end of the spring was connected to the top of the cage and allowed for 360° free rotation. All incisions were filled with xylocaine jelly and Bacitracin (McKesson, San Francisco, CA) to desensitize the surgical area and to prevent infection, respectively. At the time of cannula placement, 50% of the rats were ADX and were given 100 µg of corticosterone (in 100% alcohol, 100 µl sc) after surgery. We have found previously that >50% of rats receiving surgery for cannulation and ADX die overnight after recovering from anesthesia. This dose of corticosterone promotes 100% survival. Sham ADX was performed in the other 50% of the rats. These rats were given vehicle injections (100% alcohol). All rats were maintained in a controlled temperature and humidity environment within the University of Maryland Veterinary Care Facility with a 12:12-h light-dark cycle. These experiments were approved by the Institutional Animal Care and Use Committee of the University of Maryland at Baltimore.

The rats were allowed at least 4 days to recover. All rats had access to food and water ad libitum. All rats were fasted for 24 h. Rats were weighed and moved to the laboratory the day before the experiment. On the morning of the experiment, the water bottles were removed. Both sham and ADX rats were used in each experiment. The animals were never handled or stressed in any way before hemorrhage.

Response of blood flow to hemorrhage. Blood flow was determined before, and at 30 and 120 min after 15 ml·kg\(^{-1}\)·3 min\(^{-1}\) hemorrhage by using microspheres (15.5 ± 0.1 µm) according to the technique of Laughlin et al. (13) and modified by Darlington et al. (5). Microspheres labeled with either 85Sr, 46Sc, or 141Ce (NEN-DuPont, Wilmington, DE) were injected (200,000–300,000 spheres/injection) in 1 ml...
saline with 0.01% Tween 80. The injectate was kept in a sonication bath for at least 1 h and then vortexed for 1 min before injection. The isotope was selected randomly for each injection. Ten seconds before the injection, blood was withdrawn from the femoral cannula by a syringe pump (0.8 ml/min) for 1 min. The syringe acted as reference organ of known flow to be used in the calculation of organ blood flow (OBF; Refs. 5 and 13). Microspheres were injected into the root of the aorta over 20 s, followed by a 0.5 ml saline flush as previously described (5). The injection and flush occurred within the 1-min period. At the conclusion of the experiment, all rats were killed by pentobarbital sodium overdose (100 mg/kg ip) and their organs were removed and weighed. The stomach and intestines were cleared of digested food and feces before weighing.

The radioactivity of the organs was counted [counts per minute (CPM)] in a gamma counter (Minaxi Auto-Gamma 5000; Packard, Downers Grove, IL). OBF was determined by the equation: OBF = (CPM in organ/CPM reference organ) \times 0.80 ml/min. Backscatter ratios were determined for each isotope and were used to correct the isotope counts as described by Heymann et al. (12). Adequate mixing was assessed in each experiment by demonstrating that blood flow to the left and right kidneys was within 10% of each other as described by Heymann et al. (12) and by Laughlin et al. (13). Rats were eliminated from the experiment if inadequate mixing occurred. In this study, the percent difference between left and right kidneys was <10% before and at 30 and 120 min after hemorrhage [in sham (n = 9), 5.02 ± 1.18, 6.91 ± 2.15, and 5.62 ± 2.0%, respectively; and in ADX (n = 7), 7.33 ± 1.88, 5.10 ± 1.16, and 7.89 ± 1.54%, respectively]. Activity of the microspheres was determined to be 4.2, 4.4, and 4.6 CPM/sphere for Ce, Sr, and Sc respectively. Heymann et al. (12) have shown that the number of spheres captured by an organ has to be at least 384 for statistical relevance in the calculation of blood flow. Therefore, organs were eliminated from the study if the CPM/organ was <2,000. OBF was divided by the weight of the organ and expressed as milliliters of flow per gram of tissue. Hepatic portal blood flow was calculated from the sum of the flows to stomach, small intestines, cecum, colon, pancreas, and spleen and was divided by the weight of the liver. The forebrain was defined as being all brain structures rostral to the midcollicular level. The brainstem was caudal to the midcollicular cut minus the cerebellum. Arterial blood pressure and heart rate were measured throughout the experiment with a Cobe pressure transducer (Cobe Laboratories, Lakewood, CO) connected to a Micro-Med blood-pressure analyzer (Micro-Med, Louisville, KY). Vascular resistance was calculated as the mean arterial blood pressure divided by the blood flow of each organ.

Measurement of blood volume. Blood volume was determined by the dilution of 125I-albumin (ICN Biomedicals, Costa Mesa, CA) in the vascular space. Then 10 µCi of 125I-albumin were injected via the femoral artery 5 min before hemorrhage and 100-µl blood samples were taken before hemorrhage and 5, 10, 30, 60, 90, and 120 min after hemorrhage. Blood volume was calculated by dividing the CPM of the 100-µl sample by the CPM of the total injectate and multiplying by 10 to give milliliters of blood volume at each time point.

Statistics. Two-way analysis of variance (ANOVA) corrected for repeated measures over time was used to determine differences between sham and ADX rats in the responses of arterial blood pressure, heart rate, blood flow, vascular resistance, and blood volume. One-way ANOVA corrected for repeated measures over time was used to determine significant changes in individual flows and vascular resistance. The Newman-Keuls post hoc test was used to determine differences between groups at various time points if group effects were significant after ANOVA. Student’s t-test was used to determine differences in prehemorrhage blood volume, blood flow, and vascular resistance in various vascular beds between sham and ADX rats. Significant differences are indicated by P < 0.05.

**RESULTS**

Hemorrhage of 15 ml·kg⁻¹·3 min⁻¹ led to a rapid and significant fall in arterial blood pressure followed by a partial recovery in both sham and ADX rats (Fig. 1). However, the partial recovery in the ADX group deteriorated after 30 min and was significantly lower than in sham controls. The response of mean arterial blood pressure was significantly different between sham and ADX groups. The responses of heart rate were not different between sham and ADX rats, although heart rate tended to fall after 30 min (Fig. 1). Basal heart rate was significantly elevated in the ADX group.

ADX did not significantly affect basal blood flow to most organs studied except the arterial blood flow to the lung and liver (Figs. 2–6). Hemorrhage of 15 ml·kg⁻¹·3 min⁻¹ led to a greater fall in blood flow in ADX rats at 30 and 120 min in almost all organs studied, including the stomach, small intestines, cecum, colon, spleen, hepatic artery, portal vein, kidney, testis, lung, bone, fat, forebrain, cerebellum, and brainstem.

![Graph](http://jap.physiology.org/content/328/23/1649/F1.large.jpg)  
*Fig. 1.* Responses of mean arterial blood pressure (MABP) and heart rate in beats per min (bpm) after 15 ml·kg⁻¹·3 min⁻¹ hemorrhage in sham (○, n = 9) and adrenalectomized (ADX) rats (●, n = 7). Values represent means ± SE. *P < 0.05 for each time point by Newman-Keuls test after two-way analysis of variance (ANOVA) corrected for repeated measures over time.
stem. Also, ADX rats showed a greater increase in vascular resistance in all vascular beds except the hepatic artery and brain. In sham rats, blood flow to the forebrain, cerebellum, and brainstem did not significantly change after hemorrhage (one-way ANOVA). This suggests that cerebral tissue autoregulates to maintain flow. However, cerebral flow fell significantly after hemorrhage in ADX rats (one-way ANOVA), and the flow was significantly lower than flow in sham rats (Fig. 6). Basal hepatic arterial flow was significantly elevated in ADX rats and fell after hemorrhage. This contrasts with the response in sham rats in which hepatic arterial flow increased after hemorrhage, possibly due to autoregulation resulting from a decrease in hepatic portal flow.

Resting blood volume was not different between groups as measured with $^{125}$I-albumin ($5.96 \pm 0.36$ vs. $5.74 \pm 0.30$ ml/100 g body weight, sham vs. ADX, respectively). Hemorrhage of 15 ml·kg$^{-1}$·3 min$^{-1}$ led to a significant fall in blood volume in both sham and ADX rats (Fig. 7). In sham rats, ~30% of the shed blood was restored 5–10 min after the beginning of the 3-min hemorrhage. Restitution was partial by 1 h and was nearly complete by 2 h (Fig. 7). In ADX rats, restitution of blood volume was significantly less than that in sham rats. Blood volume was partially restored by 30 min in ADX rats. However, this restored volume fell at 2 h to levels that were significantly lower than those at 30 min (one-way ANOVA followed by Newman-Keuls post hoc test).

**DISCUSSION**

Stressors that are normally nonlethal in adrenal-intact animals can lead to cardiovascular collapse and death in adrenal insufficient animals. In this study, hemorrhage resulted in a fall, followed by a partial recovery, of arterial blood pressure in both sham and ADX rats. However, in ADX rats, after the initial recovery, arterial blood pressure fell and resulted in death. We have proposed that the cardiovascular collapse may result from either a fall in total peripheral resistance (vasodilatation) or from a failure to restore vascular volume. The results of this study show that hemorrhage of 15 ml/kg leads to an increase in vascular resistance.
resistance in most vascular beds of ADX rats. Thus the fall in arterial blood pressure that is associated with cardiovascular collapse and mortality does not result from vasodilation but most likely results from the inability of ADX rats to restore blood volume (Fig. 7). Furthermore, the initial recovery of arterial blood pressure most likely results from vasoconstriction and a partial restitution of blood volume, and the final fall in arterial blood pressure most likely results from a failure to restore blood volume coupled with a loss of the partially restored volume (Fig. 7).

ADX rats show a greater fall in blood flow and greater rise in vascular resistance in most of the organs studied (Figs. 2–6) except in the brain, where the greater fall in blood flow resulted from a drop in blood pressure as vascular resistance did not significantly change (one-way ANOVA). In sham rats, brain blood flow remained constant or increased slightly after hemorrhage. This suggests that cerebral tissue autoregulates to maintain flow. Unlike cerebral flow in sham rats, cerebral flow in ADX rats is persistently low after hemorrhage. This difference suggests that the ability to autoregulate cerebral flow is compromised. In this model, the inability to autoregulate cerebral flow may be caused directly by ADX or by the combination of ADX and occlusion of the right carotid artery, because this artery was used to deliver the microspheres. In sham rats, hepatic artery flow rises significantly after hemorrhage, possibly to compensate for the fall in hepatic portal flow (Fig. 3). This pattern is different in ADX rats, in which hepatic arterial flow is initially higher and hemorrhage leads to a fall in both hepatic arterial and portal flows. Because the temporal pattern in sham and ADX rats is different for flow to the liver and brain, it is possible that reduced flow to these organs may contribute to mortality in ADX rats after hemorrhage.

Hemorrhage in conscious ADX rats leads to cardiovascular collapse and death, although this result can be avoided by chronic corticosterone treatment (3). However, mortality is not completely prevented by infusions of corticosterone that mimic the rise in corticosterone due to hemorrhage (3); this suggests that, to prevent mortality, the endogenous glucocorticoid must be pres-
ent before the stress is applied. Pirkle and Gann (15) have previously shown that ADX dogs do not restore blood volume after hemorrhage unless given physiological glucocorticoid treatment. These authors also demonstrated that the restitution of blood volume coincided with a rise in plasma osmolality that was glucocorticoid dependent (9, 15, 17), and they suggested that extracellular solute has to be mobilized to move fluid into the vascular space. In the conscious rat model, osmolality also rises after hemorrhage, thus confirming these findings (3). However, in conscious ADX rats, the final stage of cardiovascular collapse is marked by a fall in plasma osmolality, Na\(^+\), and glucose (3, 8). These results suggest that solute is not mobilized, leading to impaired restoration of blood volume.

Restitution of blood volume after hemorrhage involves the movement of extravascular fluid into the vascular space. This is caused by 1) a fall in capillary hydrostatic pressure that results from a fall in arterial blood pressure and reflex vasoconstriction of secondary and tertiary arterioles and 2) a mobilization of solute. As capillary hydrostatic pressure falls, fluid moves into the vascular space from the interstitium in accordance with the mechanisms described by Starling (9, 10, 16). Glucose and other osmotic agents are mobilized in the interstitium and plasma, and fluid is drawn from intracellular sources into the interstitium and plasma (9, 10, 14, 17). Because ADX rats vasoconstrict to a greater degree than sham controls, the failure to restore blood volume probably does not result from an inability to decrease capillary hydrostatic pressure. It is more likely that the failure to restore blood volume is due to an inability to mobilize solute because plasma osmolality, Na\(^+\), and glucose fall just before death in ADX rats (3, 8). The gradual and progressive fall in blood volume after 30 min in ADX rats suggests that fluid is moving out of the vascular space.

ADX rats show a potentiated rise in plasma arginine vasopressin, oxytocin, renin, and catecholamines. This rise suggests that the humoral regulation of the vasculature is attempting to compensate for the fall in blood pressure (7). The initial rise in arterial blood pressure may be due to these vasoactive hormones. However, the subsequent fall of arterial pressure persists even though hormone levels remain high. This suggests that the vasculature has become insensitive to these vasoactive agents, as has been previously proposed (1, 2, 11, 18). However, in conscious ADX rats, we have found that the vascular sensitivity to vasopressin and angiotensin II is only slightly attenuated after hemorrhage, and their vascular sensitivity to the \(\alpha\)-agonist phenylephrine is not different from the sensitivity of sham controls (8). This suggests that the effect of sympathetic stimulation in the conscious ADX rat is intact and that the effects of the vasopressin and renin-angiotensin systems are only somewhat compromised. Coupled with the fact that the rise in these vasoactive hormones is potentiated after hemorrhage (7), the degree of vasoconstriction seen in this study suggests that the system is compensating for any deficit, however small, in vascular sensitivity. Indeed, most vascular beds (Figs. 2–6) showed a significantly greater increase in resistance. This result suggests that the vasculature is responding to the elevated vasopressin, renin-angiotensin, and catecholamines (3, 7).

In summary, in ADX rats, hemorrhage of 15 ml/kg body weight led to a greater decrease in blood flow and an increase in vascular resistance of most vascular beds studied. It is notable that blood flow to the brain...
and liver fell after hemorrhage in ADX rats, whereas flow to these organs remained constant or was slightly elevated in sham rats. Also, there was no restoration of blood volume by 2 h after hemorrhage in ADX rats, whereas sham rats showed complete restoration. These data suggest that the cardiovascular collapse and fall in arterial blood pressure that occurs after hemorrhagic stress in ADX rats is not caused by vasodilatation but may result from an inability to restore blood volume. Furthermore, the decrease in blood flow to the brain and liver may contribute to mortality.

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