PAPER

Mechanism of Adrenal Insufficiency Following Trauma and Severe Hemorrhage

Role of Hepatic 11β-Hydroxysteroid Dehydrogenase

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Background: Although adrenal insufficiency may not occur with moderate hypotension, it does occur with severe hemorrhage. Since hepatocellular function is depressed following severe hemorrhage, it remains unknown whether the liver plays any role in regulating adrenal function after trauma and hemorrhagic shock.

Hypothesis: Hepatic 11β-hydroxysteroid dehydrogenase (11β-HSD), a microsomal enzyme responsible for the degradation of bioactive corticosterone, plays a major role in the development of adrenal insufficiency following trauma and severe hemorrhage.

Design, Interventions, and Main Outcome Measures: Male rats underwent laparotomy to induce trauma before hemorrhage. They were then bled to and maintained at a blood pressure of 40 mm Hg until 40% of the maximal bleed-out volume was returned in the form of Ringer lactate. The rats were then resuscitated with 4 times the volume of maximal bleed-out with Ringer lactate during a 60-minute period. Plasma levels of corticosterone and corticotropin were measured at various intervals. In additional groups, corticotropin-induced corticosterone release, adrenal contents of corticosterone and cyclic adenosine monophosphate (cAMP), hepatic 11β-HSD activity, and plasma levels of corticosterone-binding globulin were determined at 1.5 hours after resuscitation. Moreover, a model of moderate hypotension (blood pressure, 80 mm Hg) was used to determine whether adrenal function is depressed under such conditions.

Results: At the time of maximal bleed-out, plasma corticosterone and corticotropin levels increased by 245% ($P<.001$) and 293% ($P<.001$), respectively. Despite corticotropin levels being similar to those of the animals undergoing sham operation after resuscitation, corticosterone levels in hemorrhaged animals remained elevated up to 4 hours after resuscitation (by 158%-207%; $P<.001$). In addition, corticotropin-induced corticosterone release decreased by 78% at 1.5 hours after resuscitation ($P = .009$). In contrast, moderate hypotension did not reduce corticotropin-induced corticosterone release. Adrenal corticosterone content and cAMP levels (ie, the second messenger of corticotropin action) decreased by 55% ($P<.001$) and 25% ($P = .03$), respectively. Hepatic 11β-HSD activity decreased significantly at 1.5 hours after resuscitation ($P<.001$).

Conclusions: Sustained increase in plasma corticosterone levels following hemorrhage and resuscitation may be, in part, due to the decreased hepatic 11β-HSD activity. The high level of corticosterone negatively regulates corticotropin release, further reducing adrenal responsiveness to corticotropin stimulation. Thus, the liver appears to play an important role in regulating adrenal function following trauma and severe hemorrhage.

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DESPITE MAJOR advances in the management of trauma, a large number of trauma patients subsequently die of the ensuing sepsis, septic shock, and multiple organ failure.[1-3] Although early and rapid fluid resuscitation remains the cornerstone of the treatment of trauma victims, studies have indicated that hepatocellular dysfunction (ie, the depressed clearance of indocyanine green), which occurs early after hemorrhage, persists despite crystalloid resuscitation.[4,5] In addition, circulating levels of liver enzymes (alanine aminotransferase and aspartate aminotransferase) increase significantly following trauma and hemorrhagic shock,[6] indicating hepatocyte damage under such conditions. Although adrenal insufficiency occurs frequently during sepsis and septic shock,[7-9] and although administration of hydrocortisone in patients with sepsis has been reported to reverse the altered hemodynamics during late septic shock,[10] it is a common notion that adrenal insufficiency occurs rarely after trauma and surgery.[11,12] However, recent studies by Barquist and Kirton[13] have indicated that although the overall inci-

MATERIALS AND METHODS

ANIMAL MODEL OF TRAUMA AND SEVERE HEMORRHAGE

We used a nonheparinized model of trauma-hemorrhage and resuscitation in the rat, as described previously,[5] with minor modifications. Briefly, male Sprague-Dawley rats (Charles River Laboratory, Wilmington, Mass) weighing 275 to 325 g were not fed for 16 hours before the experiment but were allowed water ad libitum. The animals were anesthetized using methoxyflurane inhalation; they underwent a 5-cm ventral midline laparotomy to induce tissue trauma before the onset of hemorrhage. The abdominal incision was then closed in layers. Both femoral arteries were cannulated using polyethylene-50 tubing for bleeding or monitoring of mean arterial pressure. All incisions were then closed and bathed with 1% lidocaine hydrochloride to provide analgesia throughout the experiment. The animals were then bled to a mean arterial pressure of 40 mm Hg (ie, severe hypotension) within 10 minutes. The blood pressure of 40 mm Hg was maintained by removing more blood until the animal was no longer able to maintain its blood pressure at that level (ie, maximal bleed-out). At that point, the blood pressure was further maintained by returning fluid in the form of Ringer lactate solution (RL) intravenously until 40% of the shed blood volume was returned in that form. Following this, the animals were resuscitated with RL at 4 times the volume of maximal bleed-out during a period of 60 minutes. Animals undergoing sham operation (hereafter referred to as sham animals) underwent the same surgical procedure but were not bled or resuscitated. The time required for maximal bleed-out was approximately 45 minutes, the volume of maximal bleed-out was approximately 60% of the calculated circulating blood volume,[17] and the total hemorrhage time was approximately 90 minutes. Blood samples were collected at various time points for measurement of plasma corticosterone and adrenocorticotropic hormone (corticotropin) levels. In additional groups of animals, adrenal glands were harvested at 1.5 hours after the completion of hemorrhage and resuscitation for determination of tissue corticosterone and corticotropin levels. Moreover, corticotropin-induced release of corticosterone was assessed at 1.5 hours after resuscitation in separate groups of animals. There were 6 to 8 rats in each group at each time, and the rats were killed using an overdose of intravenous pentobarbital sodium at the end of each experiment. The experiments described herein were performed in adherence to the National Institutes of Health guidelines for the use of experimental animals. This project was approved by the Institutional Animal Care and Use Committee of Rhode Island Hospital, Providence.

EXPERIMENTAL MODEL OF MODERATE HYPOTENSION

To determine whether adrenal insufficiency occurs only following severe hemorrhagic shock, a model of moderate hypotension was also used in additional groups of animals. Briefly, male Sprague-Dawley rats (275-325 g) were not fed overnight before the experiment but were allowed water ad libitum. The rats were anesthetized using methoxyflurane inhalation before the induction of trauma (ie, 5-cm midline laparotomy). Following cannulation of various blood vessels, the rats were bled to and maintained at a mean arterial pressure of 80 mm Hg for 90 minutes (moderate hypotension). The animals were then resuscitated using RL at 4 times the volume of the withdrawn blood (5.46 ± 0.45 mL/rat; approximately 30% of the calculated blood volume) during a 60-minute period. Blood samples were taken at various times for measuring corticosterone and corticotropin levels. At 1.5 hours after resuscitation, corticotropin-induced corticosterone release was determined in additional groups of animals. There were 5 to 8 rats in each group at each time.

DETERMINATION OF CORTICOSTERONE AND CORTICOTROPIN LEVELS

Blood samples for corticosterone and corticotropin assays were collected at 2 to 4 PM, when plasma levels were similar to the 24-hour average.[15] Plasma levels of corticosterone and corticosterone contents in the adrenal tissues were determined using a commercially available double-antibody radioimmunoassay kit specifically for rat corticosterone (Immuchem; ICN Biomedicals Inc, Costa Mesa, Calif). Plasma samples (10 µL each) were assayed in duplicates. The cross-reactivity of the radioimmunoassay for rat corticosterone was 100%. For other steroids, the cross-reactivity was as follows: desoxycorticosterone, 0.34%; testosterone, 0.10%; cortisol, 0.05%; aldosterone, 0.03%; progesterone, 0.03%; and all other tested steroids, less than 0.01%. Plasma levels of corticotropin were measured using a specific radioimmunoassay kit from Peninsula Labs

Continued on next page

dence of adrenal insufficiency was less than 1% in surgical intensive care units, 11% of the patients older than 55 years and with intensive care unit stays of 14 days or longer were found to have adrenal insufficiency. It is also reported that postoperative adrenal insufficiency is more common in patients than currently recognized.[14] Thus, investigation of the mechanism responsible for adrenal insufficiency following trauma and hemorrhage is important for improved management of severe trauma.

The enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD) is expressed extensively in the liver[15] and catalyzes the conversion of 11β-hydroxysteroids—cortisol in humans and corticosterone (the main glucocorticoid in rodents) in the rat—into their inactive forms, cortisone and 11-dehydrocorticosterone, respectively.[16] Although previous studies have demonstrated that hepatocellular dysfunction occurs early after the onset of hemorrhage and persists even after fluid resuscitation,[4] it remains unknown whether the depressed hepatocellular function is associated with a decrease in 11β-HSD activity. Our aim, therefore, was to determine whether hepatic 11β-HSD activity is reduced following hemorrhage and resuscitation and, if so, whether the decreased 11β-HSD activities are associated with adrenal insufficiency under such conditions.

(Belmont, Calif). Briefly, a 1.5-mL blood sample was collected into a polypropylene tube containing ethylenediaminetetraacetic acid (1 mg/mL) and aprotinin (500 KIU/mL) at various times during and after hemorrhage, and plasma was separated. The extraction of corticotropin was performed using columns packed with C18 sorbent. The corticotropin was eluted with 60% acetonitrile and 1% trifluoroacetic acid. Eluates were evaporated to dryness using a centrifugal concentrator. Rat corticotropin assay has less than 0.1% cross-reactivity with other tested peptides.

CORTICOTROPIN-STIMULATED RELEASE OF CORTICOSTERONE

At 1.5 hours after the completion of hemorrhage and resuscitation or a corresponding time in the sham animals, 100 µg of porcine corticotropin (1-39; 90 IU/mg; Sigma-Aldrich Corporation, St Louis, Mo) in 0.2-mL isotonic sodium chloride solution vehicles was injected intravenously.[18] Blood samples were taken before and 30 minutes after corticotropin administration for corticosterone assays, as described. Studies have indicated that the plasma concentrations of corticosterone increased rapidly following corticotropin administration, reaching a peak at 15 to 30 minutes and returning to basal levels by 90 minutes.[19] Our preliminary experiments indicated that plasma corticosterone levels reached a peak 30 minutes after intravenous injection of porcine corticotropin in sham animals (data not shown).

MEASUREMENT OF CYCLIC ADENOSINE MONOPHOSPHATE LEVELS

To determine the levels of cyclic adenosine monophosphate (cAMP) in the adrenal tissue, both adrenal glands were homogenized at 4°C with isotonic sodium chloride solution (1 mL of homogenate). The cAMP levels were determined radioimmunologically according to the manufacturer's instructions (a nonacetylated-procedure, cAMP radioimmunoassay kit; Du Pont/NEN, Boston, Mass), as described previously.[20]

DETERMINATION OF HEPATIC 11β-HSD ACTIVITY

Hepatic 11β-HSD activity was determined by assessing the rate of conversion of cortisol to cortisone.[21] Briefly, approximately 0.2 g of hepatic tissue was homogenized in 4 mL of ice-cold sodium phosphate buffer (10 mmol/L; pH, 7.0) containing 0.25-mol/L sucrose. Each assay tube contains approximately 150 000 counts per minute tritiated cortisol (Du Pont/NEN), 1.0 µmol/L nonradioactive cortisol, and 250-µmol/L β-nicotinamide adenine dinucleotide phosphate. Sodium phosphate buffer (0.1 mol/L) was added to bring the volume up to 0.4 mL. After 10-minute incubation at 37°C, 100 µL hepatic homogenate containing 1.0 mg of protein was added. After further incubation for 30 minutes, the reaction was arrested by rapidly transferring the tubes onto ice. The steroids were then extracted with 4 mL ethyl acetate containing a 40-µg mixture of nonradioactive cortisol and cortisone as carrier steroids. The extracts were dried, and the residues were resuspended in 100 µL of methanol. A fraction of the resuspension was spotted on a thin layer chromatography plate, which was developed in chloroform-methanol (volume-volume ratio, 9:1). The bands containing the labeled cortisol and cortisone were identified by UV light of the cold carriers, cut out into scintillation vials, and counted. The conversion rate of cortisol to cortisone was calculated from the specific activity of the labeled cortisol and the radioactivity of cortisone, and the results are expressed as the amount of cortisone (picomoles) formed per milligram of protein in 30 minutes.

DETERMINATION OF CORTICOSTERONE-BINDING GLOBULIN AND PLASMA PROTEIN LEVELS

Plasma levels of corticosterone-binding globulin (CBG) were assayed by using a saturation analysis.[22] Briefly, endogenous steroids were removed from the samples by dilution (1:100) in a slurry of dextran-coated charcoal (DCC). After the centrifugation to remove DCC, aliquots (100 µL) of the supernatant were incubated with 10-nmol/L tritiated cortisol (Du Pont/NEN) in the presence or absence of 2-µmol/L unlabeled cortisol. Separation of CBG-bound steroid was achieved by incubation for 10 minutes at 0°C with 600 µL of DCC, followed by centrifugation. The supernatants were subjected to liquid scintillation counting. Plasma levels of protein were measured according to the method of Lowry et al.[23]

STATISTICAL ANALYSIS

One-way analysis of variance and Tukey test or Student *t* test were used, and the differences were considered significant at $P \leq .05$. Results are presented as mean ± SEM.

RESULTS

ALTERATIONS IN PLASMA LEVELS OF CORTICOSTERONE AND CORTICOTROPIN

As shown in **Figure 1**, plasma levels of corticosterone increased by 245% ($P<.001$) at the time of maximal bleed-out. The significantly increased levels of corticosterone persisted after fluid resuscitation, ie, 158% to 207% at 0 to 4 hours after the completion of fluid resuscitation ($P<.001$) (Figure 1). Similar to the changes in plasma corticosterone levels, corticotropin levels increased by 293% ($P<.001$) at the time of maximal bleed-out (**Figure 2**). However, circulating levels of corticotropin decreased by 52% to 58% ($P<.001$) at 0 to 4 hours after the completion of fluid resuscitation, compared with the levels at the time of maximal bleed-out (Figure 2). In addition, these levels were not significantly different from the values in sham animals (Figure 2).

CORTICOTROPIN-INDUCED CORTICOSTERONE RELEASE AND ADRENAL CONTENTS OF CORTICOSTERONE AND cAMP

At 30 minutes after the intravenous administration of porcine corticotropin, plasma levels of corticosterone increased by 101% in sham animals (from 4.31 ± 0.70 to 8.68 ± 0.98 ng/mg protein; $P = .005$) (**Figure 3**). At 1.5

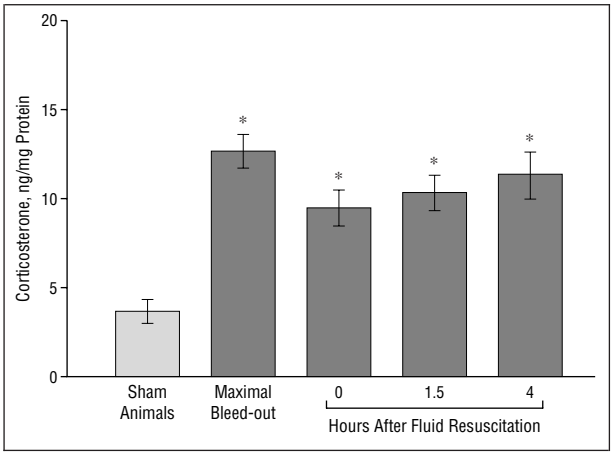

Figure 1. *Alterations in plasma levels of corticosterone in animals undergoing sham operation (sham animals), at the time of the maximal bleed-out, and 0, 1.5, and 4 hours after the completion of fluid resuscitation. Data are presented as mean ± SEM and compared using 1-way analysis of variance and Tukey test. Asterisk indicates* P<*.05 vs sham animals.*

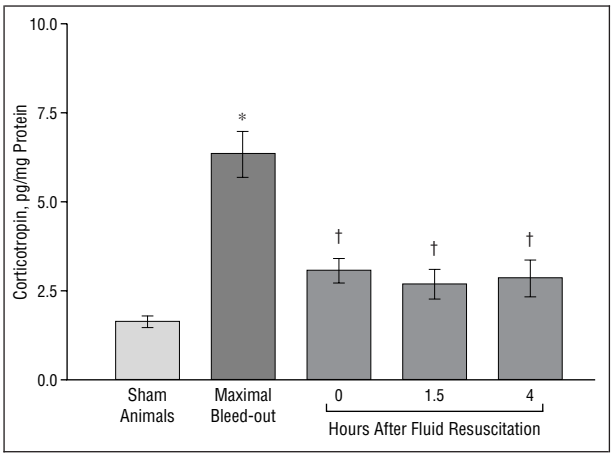

Figure 2. *Alterations in plasma levels of adrenocorticotropic hormone (corticotropin) in animals undergoing sham operation (sham animals), at the time of the maximal bleed-out, and 0, 1.5, and 4 hours after the completion of fluid resuscitation. Data are presented as mean ± SEM and compared using 1-way analysis of variance and Tukey test. Asterisk indicates* P<*.05 vs sham animals; dagger,* P<*.05 vs time of maximal bleed-out.*

hours after hemorrhage and resuscitation, however, corticotropin-induced release of corticosterone only increased by 8% (from 12.36 ± 0.96 to 13.34 ± 0.59 ng/mg protein; *P* = .41) (Figure 3). Thus, corticotropin-induced net increase of corticosterone levels was reduced by 78% from 4.37 ± 0.73 to 0.98 ± 0.75 ng/mg protein (*P* = .009). In addition, adrenal corticosterone levels decreased by 55% at 1.5 hours after hemorrhage and resuscitation (*P*<.001) (**Figure 4**, top). Similarly, the basal cAMP levels in the adrenal tissue decreased significantly following hemorrhage and resuscitation (Figure 4, bottom).

ALTERATIONS IN HEPATIC 11β-HSD ACTIVITY

As shown in **Figure 5**, hepatic 11β-HSD activity was 81.3 ± 2.6 pmol/mg protein in 30 minutes in sham animals. At 1.5 hours after the completion of hemorrhage and resuscitation, however, hepatic 11β-HSD levels decreased by 31% (*P*<.001) (Figure 5).

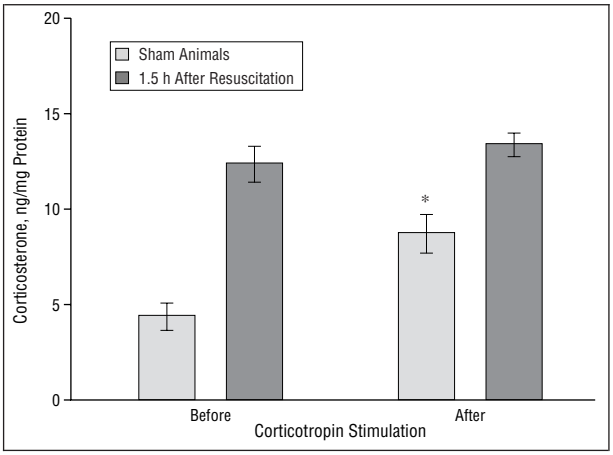

Figure 3. *Alterations in corticotropin-stimulated increase in plasma corticosterone levels in animals undergoing sham operation (sham animals) and at 1.5 hours after the completion of hemorrhage and resuscitation. Data are presented as mean ± SEM and compared using 1-way analysis of variance and Tukey test. Asterisk indicates* P<*.05 vs the respective corticosterone level before corticotropin administration.*

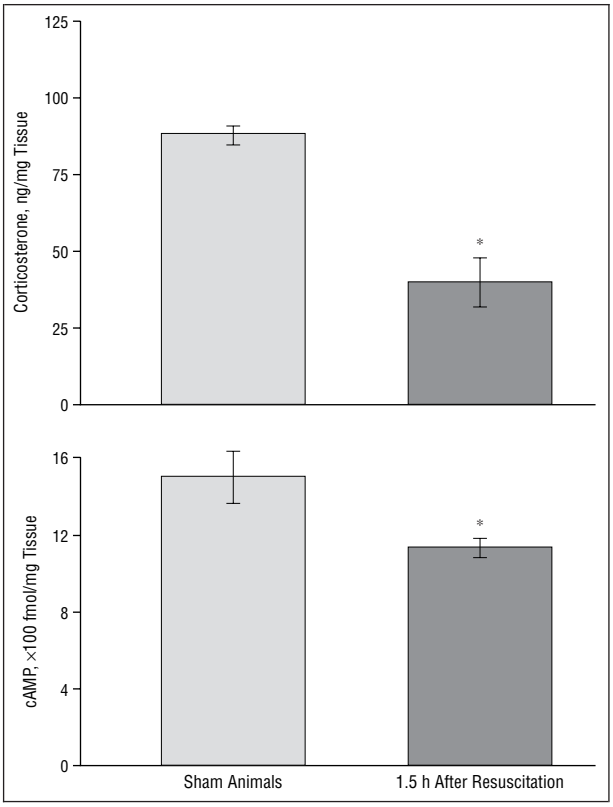

Figure 4. *Alterations in adrenal corticosterone contents (top) and adrenal cyclic adenosine monophosphate (cAMP) levels (bottom) in animals undergoing sham operation (sham animals) and at 1.5 hours after the completion of hemorrhage and resuscitation. Data are presented as mean ± SEM and compared using unpaired Student* t *test. Asterisk indicates* P<*.05 vs sham animals.*

ALTERATIONS IN PLASMA CGB LEVELS

The plasma levels of CGB were found to be 30.25 ± 2.76 pmol/mL in sham animals. Although plasma CBG levels increased to 45.50 ± 6.74 pmol/mL at 1.5 hours after the completion of hemorrhage and resuscitation, such an in-

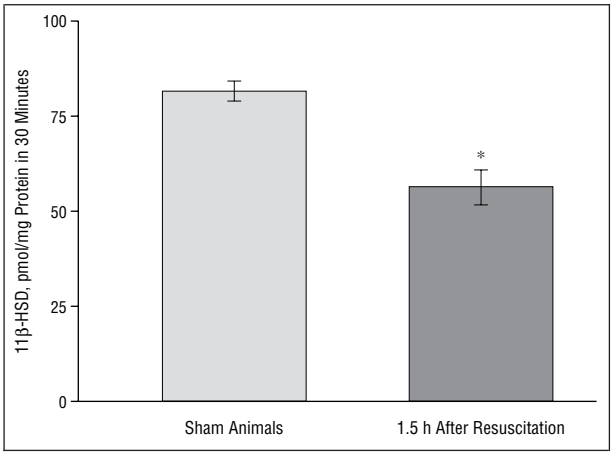

Figure 5. *Alterations in hepatic 11β-hydroxysteroid dehydrogenase (11β-HSD) activity in animals undergoing sham operation (sham animals) and at 1.5 hours after the completion of hemorrhage and resuscitation. Data are presented as mean ± SEM and compared using unpaired Student t test. Asterisk indicates P<.05 vs sham animals.*

Figure 6. *Alterations in corticotropin-stimulated increase in plasma corticosterone levels in animals undergoing sham operation (sham animals) and at 1.5 hours after the completion of hemorrhage and resuscitation in a model of moderate hypotension. Data are presented as mean ± SEM and compared using 1-way analysis of variance and Tukey test. Asterisk indicates P<.05 vs the respective corticosterone level before corticotropin administration.*

Alterations in Plasma Levels of Corticosterone and Corticotropin During and After Moderate Hypotension*

	Corticosterone, ng/mg Protein	Corticotropin, pg/mg Protein
After sham operation	3.57 ± 1.11	1.54 ± 0.16
During 45-min hemorrhage	8.22 ± 0.30†	2.39 ± 0.13†
After resuscitation, h		
0	6.63 ± 0.39†	1.67 ± 0.13
1.5	6.25 ± 0.52†	1.58 ± 0.12
4	5.81 ± 0.47	2.44 ± 0.24†

**Data are presented as mean ± SEM and compared using 1-way analyses of variance and Tukey test. Moderate hypotension indicates mean arterial pressure of 80 mm Hg for 90 minutes.*

†*P<.05 vs sham values.*

crease was not statistically significant (6 animals per group; $P = .06$).

ADRENAL RESPONSES AFTER MODERATE HYPOTENSION

Similar to the severe hemorrhage, plasma levels of corticosterone in moderate hypotension increased significantly during the hemorrhage period and persisted at 0 and 1.5 hours after the completion of fluid resuscitation (**Table**). However, the increased corticosterone levels were not statistically different from those of the sham animals at 4 hours after resuscitation (Table). Plasma levels of corticotropin were also significantly elevated during hemorrhage and at 4 hours after resuscitation (Table). Despite the similarity in the responses of corticosterone and corticotropin after moderate hypotension vs after severe hemorrhage, corticotropin induced a significant increase in corticosterone release in sham ($P = .014$) and hemorrhaged animals ($P<.001$) (**Figure 6**). The corticotropin-induced net increase of corticosterone levels was 3.81 ± 0.98 ng/mg protein in sham animals and 2.76 ± 0.32 ng/mg protein in hemorrhaged animals (reduced by 28%) ($P = .34$).

COMMENT

A number of studies have demonstrated that circulating levels of corticosterone increase following trauma, hemorrhage, and other adverse circulatory conditions.[12,13,24-30] Although it remains controversial whether administration of corticosteroids in trauma victims or patients with sepsis and septic shock is beneficial, recent studies by Bollaert et al[10] indicated that administration of modest doses of hydrocortisone in the setting of pressor-dependent septic shock for 4 days resulted in a significant improvement in hemodynamics and a beneficial effect on survival. Despite the possibility that the beneficial effects of hydrocortisone may not be directly related to the improvement in adrenocortical insufficiency,[10] it has been demonstrated that the incidence of adrenal insufficiency increases in critically ill patients at the surgical intensive care unit.[13] Furthermore, adrenal insufficiency appears to occur during the progression of multiple organ failure in trauma victims. Although adrenal insufficiency occurs after various adverse circulatory conditions such as septic shock and severe trauma or major surgery,[31] and although it has been clearly demonstrated that hepatocellular dysfunction and hepatic failure occur under such conditions,[1,4] it remains unknown whether there is any correlation between adrenal insufficiency and hepatocellular dysfunction following trauma-hemorrhage and resuscitation. Our aim, therefore, was to determine whether the hepatocellular dysfunction observed following hemorrhagic shock plays any role in the development of adrenal insufficiency.

Our results indicate that at the time of maximal bleedout, plasma levels of corticosterone and corticotropin increased significantly. However, despite the fact that corticotropin levels decreased to levels similar to those of sham animals after resuscitation, plasma corticosterone levels remained significantly elevated up to 4 hours after resuscitation. The lack of the sustained high levels of corticotropin may be due to the negative feedback of the high lev-

els of plasma corticosterone. Hepatic 11β-HSD is responsible for the degradation of circulating corticosterone in the rat. Thus, the reduced 11β-HSD activity may play a partial role in producing the sustained levels of plasma corticosterone after resuscitation. Since our results demonstrate that hepatic 11β-HSD activity was decreased significantly after hemorrhage and resuscitation, the reduction of hepatic 11β-HSD activity may be responsible for the increased levels of plasma corticosterone. The increased corticosterone levels, therefore, decreased the levels of corticotropin after resuscitation compared with the levels at the time of maximal bleed-out, through a negative feedback mechanism. Moreover, adrenal insufficiency occurred at 1.5 hours after fluid resuscitation in hemorrhaged animals, as evidenced by the decrease of corticotropin-induced corticosterone release by 78%. In addition, adrenal corticosterone content and cAMP levels (ie, the second messenger of the corticotropin action) decreased by 55% and 25%, respectively. These results, taken together, suggest that the sustained increase in plasma corticosterone levels following resuscitation may be due to the decreased hepatic 11β-HSD activity. The high level of corticosterone negatively regulates corticotropin release,[32,33] further reducing adrenal responsiveness to corticotropin stimulation. Thus, the liver appears to play an important role in regulating adrenal function following trauma and severe hemorrhagic shock.

Under normal conditions, corticotropin is secreted by the anterior pituitary gland following the stimulation of corticotropin-releasing hormone, a peptide secreted from the hypothalamus. Corticotropin then stimulates the secretion of glucocorticoid hormones, which by a negative feedback mechanism regulate release of corticotropin-releasing hormone and corticotropin.[34] The short-term effect of corticotropin on the release of corticosterone from the adrenal cortex is due to the rapid mobilization of cholesterol by binding to the membrane receptors, activating adenylate cyclase, and elevating intracellular cAMP levels. As a result, cholesterol is liberated and penetrates into the P-450 system of the inner mitochondrial membrane, thus providing more substrate for steroidogenesis within minutes.[15] In the long term (hours), corticotropin regulates the activity of the adrenal steroid hydroxylase system by elevating intracellular cAMP levels.[15] However, the corticotropin-induced corticosterone release test, which we used, appears to assess the short-term effects of corticotropin on the adrenal cortex.

Our results indicate that the basal level of adrenal cAMP decreased significantly after severe hemorrhage and fluid resuscitation. Although it remains unknown whether corticotropin-stimulated cAMP accumulation is also reduced under such conditions, our findings that corticotropin-stimulated release of corticosteroid and basal cAMP levels in adrenal tissue decreased significantly suggest that corticotropin-stimulated cAMP accumulation may have decreased. Further studies are needed to determine whether corticotropin-stimulated cAMP accumulation indeed decreases after trauma and hemorrhagic shock and, if so, whether this is due to down-regulation of corticotropin receptors, decrease of levels of adenylate cyclase and/or G proteins in the adrenal tissues, or reduction in adrenal adenosine triphosphate levels. It could be argued that the elevated levels of plasma corticosterone following severe hemorrhage may not reflect adrenal insufficiency, since a maximal corticosterone response may have been achieved without the stimulation of exogenous corticotropin. Although our study cannot rule out this possibility, other studies have clearly indicated that adrenal insufficiency occurs in combination with the elevated levels of circulating corticosterone.[7,31] In our study, the adrenal insufficiency observed after severe hemorrhage is characterized by not only the reduced corticotropin-stimulated release of corticosterone, but also the decreased adrenal contents of its second messenger, cAMP, and reduced adrenal levels of corticosterone. In addition, the occurrence of adrenal insufficiency suggests that the adrenal glands may not meet the demands for further increasing corticosterone release when a second hit (eg, sepsis) develops following trauma and hemorrhagic shock. In contrast to severe hemorrhage, adrenal insufficiency did not occur following moderate hypotension, despite significantly increased plasma levels of corticosterone under those conditions. Since 11β-HSD is responsible for degradation of circulating corticosterone, and since its activity in the hepatic tissue is reduced following trauma-hemorrhage and resuscitation, it is most likely that the reduced hepatic 11β-HSD activity is, in part, responsible for producing the elevated levels of plasma corticosterone under such conditions. In this regard, we propose that the reduced hepatic 11β-HSD activity plays an important role in maintaining the sustained high level of plasma corticosterone. The increased levels of corticosterone thus negatively regulate corticotropin-releasing hormone release from the hypothalamus and corticotropin release from the anterior pituitary gland, thereby reducing the circulating levels of corticotropin. The combination of the decreased levels of plasma corticotropin, adrenal corticosterone, and cAMP contents reduces adrenal function (ie, adrenal corticotropin responsiveness) following trauma and hemorrhagic shock. Thus, the reduced hepatic 11β-HSD activity, as the result of hepatocellular dysfunction following trauma and hemorrhagic shock, appears to have an important impact on adrenal function. Also, β-glycyrrhetinic acid is a potent inhibitor of 11β-HSD activity.[15] To further confirm the role of 11β-HSD in producing adrenal insufficiency following severe hemorrhage, β-glycyrrhetinic acid should be administered to healthy animals to determine whether the inhibition of 11β-HSD results in an elevated level of corticosterone and reduced corticotropin-induced corticosterone release.

More than 75% of the circulating corticosterone is bound to CBG under normal conditions.[15] Although it is unknown whether the ratio between bound and unbound corticosterone changes following adverse circulatory conditions in which plasma levels of corticosterone increase markedly, studies have indicated that plasma levels of CBG decrease significantly during sepsis and septic shock, but do not decrease in patients without sepsis.[34] In line with that study, we did not find plasma levels of CBG to be reduced following trauma-hemorrhage and fluid resuscitation. In contrast, the plasma CBG level was approximately 50% higher than that in sham ani-

Statement of Clinical Relevance

Despite advances in the understanding of the mechanisms responsible for cell and organ dysfunction and failure following trauma and hemorrhagic shock, a large number of trauma victims subsequently die of sepsis, septic shock, and ensuing multiple organ failure. Although adrenal insufficiency may not occur with mild trauma and moderate hypotension, it does occur following severe trauma, hemorrhagic shock, and/or major surgery. Since hepatocellular function is depressed early after hemorrhage and persists after fluid resuscitation, it is important to determine whether the depressed hepatocellular function under such conditions plays any role in the development of adrenal insufficiency. In this regard, our results indicate that the sustained increase in plasma corticosterone levels following resuscitation may be partially due to the decreased hepatic 11β-HSD activity observed under such conditions. The high level of corticosterone negatively regulates corticotropin release, further reducing adrenal responsiveness to corticotropin stimulation. Thus, the liver appears to play an important role in regulating adrenal function following trauma and severe hemorrhage. In view of this, we propose that maintenance of hepatocellular function (thereby preventing the reduction in hepatic 11β-HSD activity) may reverse the negative feedback mechanism and thereby improve adrenal corticotropin responsiveness.

mals, although such an increase was not statistically significant. It is possible that the elevated plasma CBG levels represent a compensatory mechanism that attempts to reduce the unbound corticosterone levels in the circulation. Because the measured plasma levels of corticosterone represent both bound and unbound portions of corticosterone, it remains unknown whether the increased CBG levels alter the ratio of bound and free corticosterone following trauma and hemorrhagic shock.

Our results suggest that the sustained increase in plasma corticosterone levels following resuscitation may be, in part, due to the decreased hepatic 11β-HSD activity. The high level of corticosterone negatively regulates corticotropin release, further reducing adrenal responsiveness to corticotropin stimulation. Thus, the liver appears to play an important role in regulating adrenal function following trauma and severe hemorrhagic shock.

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DISCUSSION

Herbert Hechtman, MD, Boston, Mass: The authors have revisited the question of adrenal insufficiency following trauma and hemorrhage. The data are intriguing but fall a little short of supporting a definitive conclusion. The main issue is the need to prove causality with regard to the approximately 30% decline of the hepatic dehydrogenase as a mechanism of the 3- to 4-fold rise in corticosterone. These percentage changes do not appear to be in synchrony. What would be the effect of partial or complete hepatectomy therapy, removing all or part of liver dehydrogenase activity, on adrenal function? Is the significance of corticosterone a negative regulator in the setting of trauma and hemorrhage? In sham animals, would an infusion of corticosterone, sufficient to raise circulating levels 3 to 4 times normal, not lead to a reduction of corticotropin below normal values? In contrast, corticotropin levels in your severe hemorrhage animals remain elevated to 2 times baseline.

The fascinating question is why the elevated corticosterone levels following trauma and hemorrhage lead to adrenal unresponsiveness to corticotropin in only 1½ hours. Have you shown the same phenomena by a corticosterone infusion to mimic levels seen following trauma?

Finally, you might reconsider the confusing description of these data as adrenal insufficiency, since there are high levels of adrenal hormone.

Armour Forse, MD, Boston: This is indeed an interesting paper, but a few questions are important to make it clear what degree of shock you are dealing with in this model. The model you used, particularly the severe shock, has a mortality of 50% or 60%. Could you provide us with some information about the cardiovascular parameters in this animal model? I am particularly concerned that the models not only reflect shock but also severe anemia, as the animals were resuscitated with RL. Could you give us some indication of what the hematocrit was in these animals?

Are you hypothesizing that one cause for the results was changes in hepatic function? Could you give us more information about the liver? Did you measure liver function parameters? Do you have any liver histology? Finally, did you perform any histology on the adrenal gland? This would be important in interpreting your results.

Dr Wang: In his opening remarks, Dr Hechtman questioned whether there is a cause-and-effect relationship between the decreased hepatic 11β-HSD activity and adrenal insufficiency following trauma and hemorrhagic shock. I would like to point out that the primary aim of this study was not to examine the cause-and-effect relationship, but to determine whether hepatic 11β-HSD activity is reduced following hemorrhagic shock, and, if so, whether the decreased hepatic 11β-HSD is associated with adrenal insufficiency. Our data have clearly indicated that hepatic 11β-HSD activity decreases significantly following trauma-hemorrhage and fluid resuscitation, which is associated with adrenal insufficiency. Additional studies are, however, needed to determine whether inhibition of hepatic 11β-HSD by its inhibitors, such as β-glycyrrhetinic acid, in normal animals, produces adrenal insufficiency.

Dr Hechtman suggested that we should examine the effects of partial or complete hepatectomy (ie, partial or complete removal of hepatic 11β-HSD) on adrenal function. Although studies utilizing models of partial or complete hepatectomy may provide some mechanistic insights into the role of hepatic 11β-HSD in producing adrenal insufficiency, the inhibition of 11β-HSD activity by β-glycyrrhetinic acid may be a better approach, since hepatectomy in itself will significantly affect systemic metabolisms and host defense mechanisms.

Another question of Dr Hechtman's was whether or not administration of corticosterone in normal animals reduces plasma corticotropin below normal values. Although it has been well documented that the synthesis and release of corticotropin are negatively regulated by circulating levels of corticosterone, it remains unknown whether plasma corticotropin decreases below the basal level when plasma corticosterone increases 3 to 4 times higher than the basal level. The fact that plasma corticotropin decreased significantly after fluid resuscitation as compared to the levels at the time of maximal bleedout would suggest that the sustained high levels of plasma corticosterone negatively regulate corticotropin synthesis/release following hemorrhage and resuscitation.

Dr Hechtman also raised the issue that adrenal unresponsiveness to corticotropin stimulation occurs as early as 1.5 hours after the completion of hemorrhage and resuscitation. Since hemorrhage time was approximately 90 minutes, and the time required for fluid resuscitation was 60 minutes, 1.5 hours after resuscitation represents approximately 4 hours from the onset of hemorrhage. With regard to the reduced adrenal responsiveness to corticotropin after hemorrhage, we propose this is probably due to a combination of the decrease in corticotropin receptor number and/or affinity, uncoupling between corticotropin receptor and adenylate cyclase, decrease in intracellular levels of cAMP, and reduction in the adrenal corticosterone levels. However, it remains to be determined whether administration of corticosterone in normal animals can mimic the reduced adrenal responsiveness to corticotropin which is observed following trauma and hemorrhage.

With regard to Dr Hechtman's last question, adrenal insufficiency is defined as a condition in which corticotropin-induced release of corticosterone is significantly reduced. In this study, although plasma levels of corticosterone remained significantly elevated, corticotropin-induced corticosterone release decreased by 78% at 1.5 hours after the completion of hemorrhage and resuscitation. Thus, the occurrence of adrenal insufficiency suggests that the adrenal glands will not meet the demands for further increasing corticosterone release when a second hit (eg, sepsis) develops following trauma and hemorrhagic shock.

Dr Forse's question dealt with cardiovascular and hepatic responses following trauma-hemorrhage and resuscitation. We have indeed performed various studies to determine alterations in cardiovascular and hepatocellular functions in the hemorrhage model used in this study. Our results indicate that cardiac performance, cardiac output, and organ blood flow decrease significantly after hemorrhage, despite crystalloid resuscitation. In addition, hepatocellular function, as determined by indocyanine green clearance, decreases during hemorrhage and persists following resuscitation. Our preliminary results also indicate that oxygen delivery decreases significantly following hemorrhage and resuscitation. The systemic hematocrit decreases by more than 50% following fluid resuscitation in the hemorrhaged animals. Thus, tissue hypoxia may occur in this model of hemorrhage. Although liver histology shows focal necrosis and neutrophil infiltration, we have not examined adrenal histology after trauma-hemorrhage and resuscitation.