Testosterone receptor blockade after trauma and hemorrhage attenuates depressed adrenal function

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Received 7 January 2000; accepted in final form 19 June 2000

Although the testosterone receptor antagonist flutamide restores the depressed immune function in males after trauma and hemorrhage, it remains unknown whether this agent has any salutary effects on adrenal function. To study this, male rats underwent laparotomy and were bled to and maintained at a blood pressure of 40 mmHg until 40% of the shed blood volume was returned in the form of Ringer lactate. Animals were then resuscitated and flutamide (25 mg/kg body wt) was administered subcutaneously. Plasma adrenocorticotropic hormone (ACTH) and corticosterone, as well as adrenal corticosterone and cAMP were measured 20 h after resuscitation. In additional animals, ACTH was administered and ACTH-induced corticosterone release and adrenal cAMP were determined. The results indicate that adrenal contents of corticosterone and cAMP were significantly decreased and morphology was altered after hemorrhage. Administration of flutamide improved corticosterone content, restored cAMP content, and attenuated adrenal morphological alterations. Flutamide also improved the diminished ACTH-induced corticosterone release and adrenal cAMP response at 20 h after hemorrhage and resuscitation. Furthermore, the diminished corticosterone response to ACTH stimulation in the isolated adrenal preparation was improved with flutamide. These results suggest that flutamide is a useful adjunct for improving adrenal function in males following trauma and hemorrhage.

Adrenal function; isolated perfused adrenal preparation; corticosterone; adenosine 3′,5′-cyclic monophosphate; adrenocorticotropic hormone

Despite major advances in the management of trauma victims, a large number of such patients subsequently die of ensuing sepsis, septic shock, and multiple organ failure (5, 14, 18). Although fluid replacement remains the cornerstone for the treatment of trauma victims, previous studies have indicated that cardiovascular, hepatocellular, intestinal, and renal dysfunctions occur after trauma and hemorrhage despite fluid resuscitation (33, 36–39). Moreover, depressed adrenal function is not uncommon in the clinical arena, and the incidence of adrenal insufficiency increases in critically ill patients in the surgical intensive care unit (2, 4, 6, 9, 15, 16, 26). Our recent findings also indicate that depressed adrenal function occurs early following trauma and severe hemorrhage in male animals (34). Because depressed adrenal function is usually associated with cardiovascular dysfunction and depressed function in other organs following various stresses (10, 13), including hemorrhage (12, 13), it is important to investigate potential therapeutic approaches for maintaining adrenal function following trauma and hemorrhage.

Recent studies have examined the role of sex hormones in the pathophysiology of trauma and severe hemorrhage (1, 41–43). It has been demonstrated that female animals show a normal or even enhanced immune response after trauma and hemorrhage, whereas males exhibit a depressed immune response (42). Furthermore, since gonadectomy before the induction of trauma and hemorrhage in male mice prevents the occurrence of the immune depression (43), this suggests that male sex hormones may play an important role in the regulation of posttraumatic immune responses. Moreover, flutamide, a nonsteroidal testosterone receptor antagonist, has been shown to restore the depressed immune function in males after hemorrhage (1, 41). In regard to organ functions, administration of flutamide following trauma and hemorrhage has been shown to improve the depressed cardiac and hepatic functions in male rats (28). However, it remains unknown whether this agent has any salutary effects on the depressed adrenal function in males under such conditions. Therefore, the aim of this study was to determine whether testosterone receptor blockade with flutamide following trauma and severe hemorrhagic shock attenuates depressed adrenal function under those conditions in male animals.

MATERIALS AND METHODS

Animal model of trauma and severe hemorrhage. A nonheparinized model of trauma and hemorrhage and resuscita-
tion in the rat, as previously described by us (36), was used in this study with minor modifications. Briefly, male Sprague-Dawley rats (275–325 g) were fasted overnight before the experiment but were allowed water ad libitum. The animals were anesthetized with methoxyflurane inhalation and 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 and one femoral vein were cannulated with polyethylene-50 tubing for bleeding, monitoring of mean arterial pressure, and fluid resuscitation. All incisions were closed and bathed with 1% lidocaine to provide analgesia throughout the experiment. The animals were then bled to a mean arterial pressure of 40 mmHg (i.e., severe hypotension) within 10 min. The rapid bleeding on awakening puts the animals in a state of depressed sensibility, thus minimizing distress to the animals. The blood pressure of 40 mmHg was maintained by removing more blood in increments of 0.2 ml until the animal was no longer able to keep blood pressure at that level (i.e., maximum bleedout). At that point, the blood pressure was maintained thereafter by infusing Ringer lactate intravenously in 0.2-ml bolus increments until 40% of the shed blood volume was returned in that form. After this, the animals were resuscitated with four times the volume of maximum bleedout with Ringer lactate over a period of 60 min at a constant rate. Sham-operated animals underwent the same surgical procedure but were neither bled nor resuscitated. The time required for maximum bleedout was ~45 min, the volume of maximum bleedout was ~60% of the calculated circulating blood volume (35), and the total hemorrhage time was ~90 min. 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.

Experimental protocol. At 15 min before the end of resuscitation, the rats received 25 mg/kg body weight flutamide (Schering Plough, Kenilworth, NJ) subcutaneously or an equivalent volume of 0.9% saline intravenously. Flutamide is a nonsteroidal antiandrogen that is orally active and has been shown to be effective in preventing the development of the male reproductive tract and secondary sex characteristics. Flutamide has been shown to be a potent inhibitor of androgen receptor activity in vitro and in vivo. It does not interfere with the hormonal control of adrenal and gonadal function at the doses used in this study. The catheters were then removed, the vessels ligated, and the skin incisions closed with sutures. The rats were returned to their cages and allowed food and water ad libitum. At 20 h after the completion of fluid resuscitation, the animals were reanesthetized with methoxyflurane and blood samples were collected via cardiac puncture for measurement of plasma levels of adrenocorticotropic hormone (ACTH) and corticosterone. The right adrenal gland was harvested and fixed in 10% neutral-buffered Formalin for bleeding, monitoring of mean arterial pressure, and fluid resuscitation. Adrenals were then harvested and fixed in 10% neutral-buffered Formalin for histological examination. Adrenals were then harvested and fixed in 10% neutral-buffered Formalin (Sigma) and later embedded in paraffin. The tissue was then sectioned at a thickness of 5 μm and stained with hematox-
ylin and eosin. Slides were evaluated by light microscopy and documented by photographs.

Statistical analysis. All data are presented as means ± SE. One-way analysis of variance (ANOVA) and Tukey’s test were used for the comparison among hemorrhage, hemorrhage plus flutamide, and sham animals at 20 h after resuscitation, and the differences were considered significant at $P \leq 0.05$.

RESULTS

Alterations in ACTH, corticosterone, adrenal corticosterone, and cAMP. Plasma levels of ACTH in sham-operated animals were $38.7 \pm 5.0$ pg/ml and were not significantly altered by trauma and hemorrhage, irrespective of flutamide administration (data not shown). Similarly, plasma levels of corticosterone in sham-operated animals were $230.2 \pm 30.2$ ng/ml and were not significantly different in animals undergoing hemorrhage, irrespective of flutamide treatment (data not shown). As shown in Fig. 1, adrenal levels of corticosterone in sham-operated animals were $405 \pm 30$ ng/mg protein. Adrenal corticosterone decreased by $32\%$ ($P < 0.05$) at 20 h after hemorrhage and resuscitation (Fig. 1). However, in hemorrhaged animals receiving flutamide treatment, the corticosterone levels were $346 \pm 40$ ng/mg protein, which were higher than in nontreated animals (Fig. 1). There were no significant differences between hemorrhaged animals with flutamide treatment and sham-operated animals. As shown in Fig. 2, adrenal cAMP levels in sham-operated animals were $6.54 \pm 0.25$ pmol/mg protein, which decreased by $24\%$ ($P < 0.05$) after hemorrhage. Flutamide treatment, however, increased adrenal cAMP to the level similar to sham-operated animals (Fig. 2).

ACTH-induced corticosterone release and adrenal contents of cAMP. As shown in Fig. 3, plasma levels of corticosterone increased by $281.8 \pm 32.8$ ng/ml in sham-operated animals at 30 min after the intravenous administration of porcine ACTH. At 20 h after the completion of hemorrhage and resuscitation, ACTH-induced release of plasma corticosterone was only $68\%$ of sham levels ($P < 0.05$; Fig. 3). However, in hemorrhaged animals receiving flutamide treatment the ACTH-induced release of plasma corticosterone increased by $291.7 \pm 24.8$ ng/ml ($P < 0.05$). The level of corticosterone release in the hemorrhaged and flutamide-treated animals were similar to sham levels (Fig. 3). Moreover, ACTH-induced net increase of cAMP in sham-operated animals was $39.86 \pm 2.56$ pmol/mg protein at 5 min after the intravenous administration of porcine ACTH. Flutamide treatment restored the depressed cAMP increase in hemorrhaged animals from $28.25 \pm 3.27$ to $41.12 \pm 2.14$ pmol/mg protein (Fig. 4).

ACTH-induced corticosterone release in the isolated perfused adrenal preparation. As shown in Fig. 5, corticosterone release from the isolated and in situ perfused adrenals increased by fourfold after ACTH perfusion in sham-operated animals. At 20 h after the completion of hemorrhage and resuscitation, however, ACTH-induced release of corticosterone remained similar to the basal levels (Fig. 5). Hemorrhaged animals

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**Fig. 1.** Alterations in adrenal corticosterone at 20 h after sham operation, hemorrhage (Hem), or hemorrhage with flutamide treatment (Hem + F); n = 6/group. Data are expressed as means ± SE and compared by one-way analysis of variance (ANOVA) and Tukey's test. *$P < 0.05$ vs. sham.

**Fig. 2.** Alterations in adrenal cAMP at 20 h after sham operation, hemorrhage (Hem), or hemorrhage with flutamide treatment (Hem + F); n = 6/group. Data are expressed as means ± SE and compared by one-way ANOVA and Tukey’s test. *$P < 0.05$ vs. sham. # $P < 0.05$ vs. Hem.

**Fig. 3.** Alterations in the net increase in plasma corticosterone at 0.5 h post-ACTH stimulation after sham operation, hemorrhage (Hem), or hemorrhage with flutamide treatment (Hem + F); n = 6/group. Data are expressed as means ± SE and compared by one-way ANOVA and Tukey’s test. *$P < 0.05$ vs. sham. # $P < 0.05$ vs. Hem.
receiving flutamide treatment increased ACTH-induced release of corticosterone by more than threefold, which was not significantly different from sham-operated animals (Fig. 5).

Alterations in adrenal water content and morphology. The adrenal water content was 66 and 75% in sham-operated and hemorrhaged animals, respectively. Flutamide treatment, however, reduced the water content ratio to 70% at 20 h after hemorrhage and resuscitation. The adrenal histology in sham-operated animals is presented in Fig. 6A. The zona glomerulosa and zona fasciculata of the adrenal cortex in hemorrhaged animals exhibited marked edema (Fig. 6B). Loss of staining intensity in the cytoplasm (arrow, Fig. 6B) and enlarged cell volume (arrowheads, Fig. 6B) were seen in these regions of the adrenal cortex in hemorrhage. The adrenal glands, however, demonstrated normal cell structure and reduced tissue edema in hemorrhaged animals receiving flutamide treatment (Fig. 6C).

DISCUSSION

Various stresses, including hemorrhage, are accompanied by activation of the hypothalamic-pituitary-adrenal axis, leading to an increased production of glucocorticoids by the adrenals (22, 23). Although elevated glucocorticoids may function to protect the host from a hyperactive inflammatory response, which may be deleterious to cell and organ functions, glucocorticoids also play supportive roles in maintaining vascular tone, endothelial integrity, vascular permeability, and total distribution of water within the vascular compartment and potentiate the vascular responsiveness to increased catecholamines (19). The recognition and treatment of depressed adrenal function are important because adrenal insufficiency occurs in critically ill patients and is usually associated with deleterious circulatory alterations and organ dysfunction (2, 4, 9, 10, 12, 13, 15, 16, 26). However, it has been shown that the testosterone receptor antagonist flutamide improves organ function in male rats following trauma and hemorrhage (28). We, therefore, hypothesized that testosterone receptor blockade by flutamide after trauma and hemorrhage and resuscitation will also improve the depressed adrenal function in male animals.

Our results indicate that plasma levels of ACTH as well as plasma corticosterone at 20 h posthemorrhage and resuscitation remained at sham levels, irrespective of flutamide administration. Adrenal contents of corticosterone and cAMP was significantly decreased at 20 h after trauma and hemorrhage and resuscitation in vehicle-treated animals. Furthermore, hemorrhaged animals exhibited a marked decrease in ACTH-induced corticosterone release in plasma as well as adrenal levels of cAMP. These findings are supported by our previous results, which demonstrate that adrenal function decreased as early as 1.5 h and remained depressed even at 20 h after trauma and hemorrhage (34). In flutamide-treated animals, however, the depressed adrenal levels of corticosterone and cAMP and the decreased ACTH-induced corticosterone release and cAMP were improved at 20 h following hemorrhage shock and resuscitation. Moreover, by using an in situ isolated adrenal preparation, we have demonstrated that ACTH-stimulated corticosterone release remained near the baseline level at 20 h after hemorrhage and resuscitation. Administration of flutamide in vivo following resuscitation, however, restored the depressed ACTH-induced corticosterone release in vitro. Flutamide treatment also attenuated adrenal edema. To further support these findings, our histological observations showed that the adrenal glands of hemorrhaged animals receiving flutamide treatment demonstrated normal cell structure and reduced tissue edema in the zona glomerulosa and zona fasciculata of the adrenal cortex. Taken together, our data clearly demonstrate that testosterone receptor blockade with flutamide in males following trauma and hemorrhage and resuscitation attenuates depressed adrenal function. Thus flutamide appears to be a novel and useful adjunct for improving adrenal function in males following trauma and hemorrhage.

![Fig. 4. Alterations in the net increase in adrenal cAMP at 5 min post-ACTH stimulation after sham operation, hemorrhage (Hem), or hemorrhage with flutamide treatment (Hem + F); n = 6/group. Data are expressed as means ± SE and compared by one-way ANOVA and Tukey's test. *P < 0.05 vs. sham. #P < 0.05 vs. Hem.](http://ajpregu.physiology.org/)

![Fig. 5. Alterations in ACTH-induced corticosterone response in the isolated adrenal gland after sham operation, hemorrhage (Hem), or hemorrhage with flutamide treatment (Hem + F); n = 6/group. Data are expressed as means ± SE and compared by one-way ANOVA and Tukey's test. *P < 0.05 vs. sham. #P < 0.05 vs. Hem.](http://ajpregu.physiology.org/)
Under normal conditions, corticosterone is the principal glucocorticoid secreted by the adrenal cortex in the rat (cortisol in humans). The release of corticosterone is modulated by a complex negative feedback mechanism involving the hypothalamus, pituitary, and adrenal glands (23). ACTH released from the pituitary augments adrenal secretion of corticosterone, whereas falling levels of corticosterone are associated with rising levels of ACTH. Therefore, it could be argued that the decreased adrenal contents of corticosterone after trauma and hemorrhage or restored adrenal corticosterone levels in flutamide-treated animals may be due to changes in circulating levels of ACTH. Previous studies have indicated that although plasma ACTH at the time of maximal bleedout is markedly increased, levels of ACTH are similar to those of sham-operated animals after resuscitation (34). In agreement with the findings of that study, the results of this study indicate that plasma ACTH levels were not significantly different among the three tested groups at 20 h after resuscitation. Therefore, the decreased adrenal corticosterone following trauma and hemorrhage and resuscitation and the improved levels of adrenal corticosterone in flutamide-treated animals do not appear to be correlated with alterations in plasma ACTH under such conditions. Furthermore, our results indicate that animals that were administered flutamide were able to restore the decreased ACTH-induced corticosterone responsiveness after hemorrhagic shock both in vivo and in situ. This finding indicates improved adrenal function in an isolated setting removed from factors that may affect in vivo corticosterone release. The improvement in adrenal function by flutamide after severe hemorrhage is also characterized by the restoration of cAMP levels and ACTH-induced cAMP increase following hemorrhagic shock.

It is a common notion that depressed adrenal function occurs in combination with the elevated corticosterone levels in circulation (2, 4, 11, 17, 21, 23, 26, 31, 34, 40). In previous studies, we have found that plasma levels of corticosterone after hemorrhage were elevated by 245% at the time of maximal bleedout and remained elevated up to 4 h after resuscitation (34). The elevated corticosterone in plasma may be due to decreased hepatic enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD) activity, which is responsible for corticosterone metabolism. In this study, however, plasma levels of corticosterone were similar to sham levels at 20 h after hemorrhagic shock and resuscitation, irrespective of flutamide treatment. It also could be possible that the corticosterone levels observed late after hemorrhage are the result of impaired hypothalamic or pituitary function. This may be the case because plasma levels of ACTH were not elevated at 20 h after hemorrhage despite sham levels of corticosterone. However, our data indicate that corticosterone and cAMP levels in the adrenal gland significantly decreased and the net increase in plasma corticosterone and adrenal cAMP after ACTH stimula-
tion decreased significantly following hemorrhage. This is supported by the results of the isolated adrenal preparation that corticosterone content in the effluent was significantly decreased following hemorrhage.

Flutamide is a nonsteroidal antiandrogenic agent that has been used extensively in the treatment of prostate cancer (3). This agent appears to exert its antiandrogenic effects on male secondary sex structures by the inhibition of androgen uptake and/or inhibition of nuclear binding of androgens (7). In humans, maximal plasma concentrations of unaltered flutamide are reached within 1 h of oral administration. However, concentrations of the principal active hydroxylated metabolite, 2-hydroxyflutamide, reach higher concentrations in plasma than the parent drug, and data based on limited studies indicate that the half-life of this metabolite is 4–7 h after administration (7). It is this metabolite of flutamide that appears to be largely responsible for its antiandrogenic activity. However, the precise mechanism responsible for the beneficial effects of flutamide on adrenal function after hemorrhage and resuscitation remains unknown. Previous studies have shown that plasma testosterone levels are not significantly altered early following trauma and hemorrhage and resuscitation (43). Therefore, the effect of flutamide on adrenal function may not be mediated through alterations in levels of testosterone but may be due to decreased testosterone receptor activity and its signal transduction mechanisms or enhanced specific cellular effects of other sex steroids such as estradiol. Our data indicate that flutamide treatment reduced adrenal edema and attenuated the morphological changes in the adrenals induced by hemorrhage. Recent studies have indicated that testosterone increases vascular smooth muscle thromboxane A2 receptors in the aorta (25). It also has been shown that testosterone treatment enhances vasoconstriction in response to thromboxane A2 in coronary circulation (20, 25), which can be blocked by administration of flutamide (20). Additionally, it has been shown that testosterone treatment inhibits the synthesis of prostacyclin by rat aortic smooth cells in culture (27). There is indeed evidence for the expression of androgen receptors on the adrenal cortex (30). Additionally, studies have indicated the production of prostaglandins from adrenal tissue (29). However, it remains to be determined whether inhibition of thromboxane A2 and/or the enhancement of prostacyclin is the mechanism by which flutamide increases adrenal perfusion and subsequently improves adrenal function. Another mechanism by which flutamide restores the depressed adrenal function after hemorrhagic shock could be due to improvements in hepatic function. Our previous studies have indicated that administration of flutamide following hemorrhage improves hepatocellular function (28). Because 11β-HSD is released by the liver and its activity is decreased early after hemorrhage, flutamide may have restored the ability of the liver to produce this enzyme in response to increased levels of corticosterone. Furthermore, because high levels of corticosterone negatively regulate corticosterone release by further reducing adrenal responsiveness to ACTH stimulation, the potential restoration of 11β-HSD activity by flutamide may have maintained adrenal responses to stimulation (34).

In summary, the results indicate that despite similar plasma levels of ACTH in hemorrhaged and sham animals, adrenal contents of corticosterone and cAMP were significantly decreased after hemorrhage. Administration of flutamide, however, improved the depressed adrenal corticosterone contents and restored cAMP in the adrenal gland after hemorrhagic shock. Flutamide also restored the diminished plasma corticosterone response induced by ACTH stimulation and improved ACTH-induced cAMP increase in the adrenal gland after hemorrhagic shock and resuscitation. Additionally, flutamide improved the diminished corticosterone response to ACTH stimulation in vitro, reduced water content in adrenal gland tissue, and increased the percentage of perfusate recovery at 20 h after hemorrhage and resuscitation. Thus testosterone receptor blockade using flutamide appears to be a useful approach for improving depressed adrenal function in males after trauma and severe hemorrhagic shock.

Perspectives

Despite advances in the management of trauma and hemorrhage victims, a large number of such patients die of ensuing sepsis, septic shock, and multiple organ failure, which remain major causes of morbidity and mortality in surgical intensive care units. Although fluid replacement remains the cornerstone for the treatment of trauma victims, depressed adrenal function is not uncommon in critically ill patients and the incidence of adrenal insufficiency increases in such patients in the surgical intensive care unit. Because depressed adrenal function is usually associated with cardiovascular dysfunction and depressed function in other organs after various stresses, including hemorrhage, it is important to investigate potential therapeutic approaches for maintaining adrenal function under such conditions. In this regard, the results presented in this study demonstrate that testosterone receptor blockade with flutamide in males following trauma and severe hemorrhagic shock improves the depressed adrenal corticosterone contents and restored adrenal cAMP contents. Flutamide also improves the diminished ACTH-induced corticosterone release and adrenal cAMP response in addition to attenuating morphological alterations such as edema. Furthermore, flutamide improves the diminished corticosterone response to ACTH stimulation in an isolated adrenal preparation. These results, taken together, suggest that flutamide appears to be a novel and useful adjunct for improving adrenal function in males following trauma and hemorrhage. With advances in the knowledge and treatment of cell and organ dysfunction following trauma and hemorrhage, future studies will lead to improved management of such patients and decreased morbidity and mortality.
REFERENCES


