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Unilateral adrenalectomy attenuates hemorrhagic shock-induced analgesia in rats

Taeko Fukuda, Chikako Nishimoto, Masayuki Miyabe, and Hidenori Toyooka

Department of Anesthesiology, Graduate School of Comprehensive Human Sciences, Tsukuba University, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8575, Japan

Abstract
Purpose. To assess the importance of the pituitary adrenal axis in producing stress-induced analgesia (SIA) after hemorrhagic shock, we performed formalin tests after hemorrhage and reinfusion in unilaterally adrenalectomized or sham-operated rats.
Methods. Fifty-two adult Sprague-Dawley rats were divided into seven groups: sham-operation normotensive (n = 8), sham-operation shock (n = 8), adrenalectomy normotensive (n = 7), adrenalectomy shock (n = 7), sham-operation shock + yohimbine (n = 7), sham-operation normotensive + corticosterone (n = 7), and adrenalectomy shock + corticosterone (n = 8). The left adrenal gland was cauterized 24 h before the experiment. The mean blood pressure in the shock groups was kept at 50–60 mmHg for 30 min by draining arterial blood. After the blood-reinfusion or observation period, 10% formalin was injected into the rear paw. Nociceptive behaviors and locomotion were observed and rated for 1 h, using the criteria of Dubuisson and Dennis. In 12 other sham-operated and adrenalectomized rats, plasma adrenalin, noradrenalin, and corticosterone concentrations were measured before and after hemorrhagic shock.
Results. Although the sham-operation shock group showed a lower pain score, the adrenalectomy shock group showed nociceptive behavior similar to that in the normotensive groups. Yohimbine did not affect the SIA; however, corticosterone administration reversed the effects of the adrenalectomy on the SIA. The plasma corticosterone levels in the unilaterally adrenalectomized rats were lower than those in the sham-operated rats and did not increase after hemorrhagic shock.
Conclusion. These results suggest that adrenocortical systems play an important role in hemorrhagic shock-induced SIA.

Key words Stress-induced analgesia · Hemorrhagic shock · Adrenalectomy · Formalin test

Address correspondence to: T. Fukuda
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Introduction

A wide variety of stressful events produce a reduction in reactivity to pain that has been labeled stress-induced analgesia (SIA) [1–3]. In an earlier study, we observed SIA after hemorrhagic shock [4]. It has been found that the anesthetic requirement decreased during hypovolemic shock, but our study was the first to report the SIA phenomenon under a normotensive condition after hemorrhagic shock. In our next study, we found that naloxone pretreatment did not reverse the SIA [5]. Endogenous opioids may not be the main factor governing SIA after hemorrhagic shock. The mechanisms of SIA after hemorrhagic shock are still unclear.

Surgical or functional blockade of the hypophyseal-adrenocortical system was reported to influence the induction of analgesia by the stress of cold-water swimming or electric shock [6–10]. For example, Kelly et al. [8] demonstrated that total hypophysectomy, but not posterior lobectomy, reduced antinociception induced by continuous cold-water swimming stress. Filaretov et al. [10] reported that functional blockade of the hypophyseal-adrenocortical system reduced the analgesia induced by electrical shock stress, and they noted that the attenuation occurred in parallel to plasma corticosterone levels. We speculated that the hypophyseal-adrenocortical system might mediate hemorrhagic shock-induced analgesia.

We destroyed the left adrenal gland in rats by electrocautery and examined SIA after hemorrhagic shock, using a formalin-induced pain stimulus test (formalin test). Furthermore, we also investigated the contribution of the adrenocortical or adrenomedullary system to the origin of SIA, using yohimbine or corticosterone. In addition, we measured the plasma corticosterone and catecholamine concentrations before and after hemorrhagic shock in other sham-operated and adrenalectomized rats.

Materials and methods

All experimental methods were approved by our institutional animal care committee. Fifty-two adult male Sprague-Dawley rats, weighing 300–350 g, were divided into seven experimental groups: a sham-operation normotensive (Sham-Normo) group (n = 8), a sham-operation hemorrhagic shock (Sham-Shock) group (n = 8), a unilateral adrenalectomy normotensive (Adrenal-Normo) group (n = 7), a unilateral adrenalectomy hemorrhagic shock (Adrenal-Shock) group (n = 7), a sham-operation hemorrhagic shock + yohimbine (Sham-Shock-Yohimbine) group (n = 7), a sham-operation normotensive + corticosterone (Sham-Normo-Cor) group (n = 7), and an adrenalectomy hemorrhagic shock + corticosterone (Adrenal-Shock-Cor) group (n = 8). With the animals under pentobarbital anesthesia, the left adrenal gland was destroyed by electrocautery in the three adrenalectomy groups, and a skin incision alone was done in the four sham-operation groups. An arterial catheter was placed in the right common carotid artery in all animals. We had confirmed, by means of a preliminary study (n = 20), that the ligation of this artery had no effect on the rats' behavior during the formalin test [4]. Twenty-four hours after these preparations, 8–14 ml of arterial blood was drawn, and the mean blood pressure (mBP) was kept between 50 and 60 mmHg for 30 min. The drained blood was mixed with heparin and reinfused into each rat in the four hemorrhagic shock groups. In the three normotensive groups, mBP and heart rate (HR) were checked for 30 min without any blood withdrawal. Each rat was checked for neurological abnormalities (locomotion, motor activities, vigilance ability), and arterial blood gas was analyzed 15 min after the reinfusion or observation. Yohimbine (1 mg·kg^{-1}) or corticosterone (4 mg) was administered intraperitoneally 30 min before the formalin test. The administration dose of yohimbine was based on the data of Sierralta et al. [11], which showed that the antinociceptive activity of intracerebroventricularly administered clonidine was most effectively antagonized by intraperitoneal yohimbine (1 mg·kg^{-1}). The corticosterone dose was calculated on the basis of the difference in corticosterone concentration (200 ng·ml^{-1}) between the sham-operated and adrenalectomized rats, and based on the blood volume of the rats (20 ml). The blood volume for each rat was estimated according to the Lee and Blaufox [12] regression equation for rats: blood volume (ml) = 0.06 × body weight + 0.77. Using a 26-gauge needle, 10% formalin (3.7% formaldehyde solution, 0.1 ml) was injected subcutaneously into the plantar surface of the left rear paw after the arterial blood gas analysis.

Observation of the formalin test began immediately after the formalin was injected. The rats were put in a transparent plastic chamber in which a mirror was installed so as to allow an unobstructed view of the paws. The pain behavior was observed for the first 5 min of each 10-min period between 0 min (immediately after formalin injection) and 55 min, i.e., 0–5 min, 10–15 min, and so on. The effects were rated according to the criteria of Dubuisson and Dennis [13]. The pain score was calculated by the following formula: pain score = (T1 + T2 × 2 + T3 × 3)/300, where T1, T2, and T3 are durations (in s) spent in 1, 2, and 3, respectively, during each 300-s block. The categories were: 1, limping during locomotion or resting the injected paw lightly on the floor; 2, elevation of the injected paw; and 3, licking, biting, or shaking the injected paw. The formalin test was conducted between 9 a.m. and 5 p.m. The investigators were blinded to the preparation of the animals throughout the data collection phase. The room temperature was kept at 24 ± 2°C. After observation of the formalin test, arterial blood gas was checked again. Because the rats' body temperature did not change by more than 0.1°C in our preliminary study, we did not measure the temperature in any of the rats, so as to minimize stress.

To measure the plasma catecholamine and corticosterone levels, 12 other adult Sprague-Dawley rats were divided into two groups: a sham-operation and hemorrhagic shock group (n = 6) and a unilateral adrenalectomy and hemorrhagic shock group (n = 6). After basal mBP and HR were measured, 5 ml of blood was sampled to obtain basal plasma levels of adrenalin, noradrenalin, and corticosterone. Another several milliliters of blood was drawn to keep the mBP between 50 and 60 mmHg, and 5 ml of blood was then obtained 30 min later to measure plasma catecholamine and corticosterone changes. The adrenalin and noradrenalin concentrations were measured by an automated HPLC analyzer (Tosho, Tokyo, Japan). The coefficients of variation of adrenalin and noradrenalin were 2% and 1.5%, respectively. The minimum detection range of both the catecholamines was 6 pg·ml^{-1}. Corticosterone was determined by radioimmunoassay (RIA) methods with an RIA kit (Coat-A-Count, DPC; Diagnostic Products, Los Angeles, CA, USA). The coefficient of variation of the same sample was 5.8%. The minimum detection range was 5.7 ng·ml^{-1}.

Data values were presented as means ± SD. Statistical analyses were performed using two-way analysis of variance (Bonferroni post-hoc test) for mBP, HR, blood gas analysis, and drained blood volume. The Kruskal-Wallis test was used to determine the pain intensity score. Differences between mean plasma catecholamines and corticosterone in sham-operated and adrenalectomized rats before and after hemorrhagic shock were assessed with the Student's paired t-test and an unpaired test for intergroup mean concentrations. $P < 0.05$ was considered statistically significant.

Results

The mBPs in the four hemorrhagic shock groups were significantly lower than those in the three normotensive groups during the hemorrhagic shock period. However, there was no significant difference in mBP after the blood reinfusion throughout the experiment (Fig. 1a). Heart rate did not differ significantly among the seven groups either before or immediately after the blood withdrawal. However, the Adrenal-Shock group showed a bradycardia tendency before the formalin test (Fig. 1b). No rat showed any neurological abnormality.

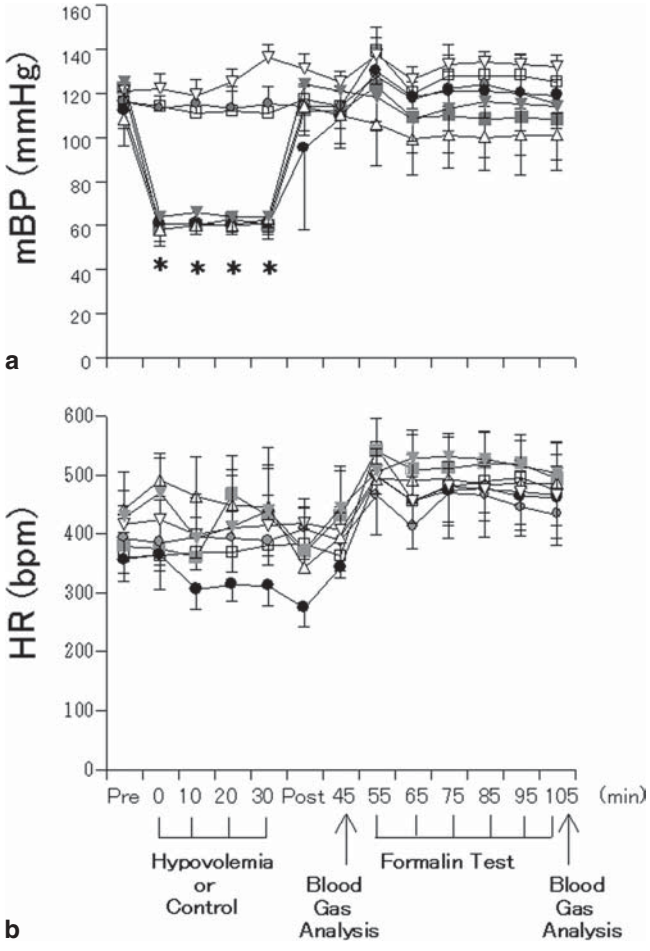

Fig. 1a,b. Time courses in the present study for **a** mean blood pressure (*mBP*) and **b** heart rate (*HR*). *Open squares*, sham-operation normotensive group (Sham-Normo); *closed squares*, sham-operation hemorrhagic shock group (Sham-Shock); *Open circles*, unilateral adrenalectomy normotensive group (Adrenal-Normo), *closed circles*, unilateral adrenalectomy hemorrhagic shock group (Adrenal-Shock), *open upward triangles*, sham-operation hemorrhagic shock + yohimbine group (Sham-Shock-Yohimbine), *open downward triangles*, sham-operation normotensive + corticosterone group (Sham-Normo-Cor); *closed downward triangles*, adrenalectomy hemorrhagic shock + corticosterone group (Adrenal-Shock-Cor). *$P < 0.05$ vs Sham-Normo, Adrenal-Normo, and Sham-Normo-Cor groups

The pain intensity scores in the Sham-Shock group were significantly lower than those in the Sham-Normo group in both the early and late phases, and the scores were significantly lower than those in the Adrenal-Normo and Adrenal-Shock groups in the late phase. The pain intensity scores in the Adrenal-Shock group did not differ from those in the Sham-Normo and the Adrenal-Normo groups in either phase. The pain intensity scores in the Sham-Shock-Yohimbine group were significantly lower than those in the Sham-Normo group; however, they did not differ from those in the Sham-Shock group in either phase. The Adrenal-Shock-Cor group showed significantly lower pain intensity scores than did the Sham-Normo, the Adrenal-Normo, the Adrenal-Shock, and the Sham-Normo-Cor groups; however, the scores in the Adrenal-Shock-Cor group did not differ from those in the Sham-Shock and Sham-Shock-Yohimbine groups (Fig. 2).

The pH level in the Sham-Normo-Cor group was slightly higher than that in the Sham-Shock-Yohimbine group before the formalin test. However, there was no significant difference in the blood gas analysis data after

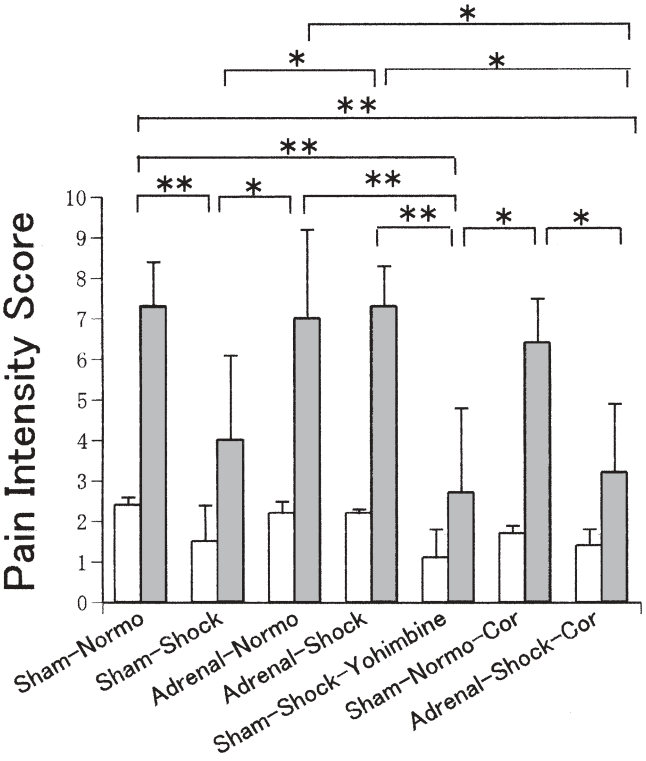

Fig. 2. Pain intensity scores in the formalin test. The *open bars* show pain scores in the early phase (0–5 min) and the *closed bars* show the sum of pain scores in the late phase (20–25, 30–35, 40–45, and 50–55 min). *$P < 0.05$, Significant difference in only the late phase; **$P < 0.05$, Significant difference in both early and late phases

Table 1. Blood gas analysis before and after the formalin test

	Sham-Normo	Sham-Shock	Adrenal-Normo	Adrenal-Shock	Sham-Shock-Y	Sham-Normo-C	Adrenal-Shock-C
n	8	8	7	7	7	7	8
Before the formalin test							
pH	7.49 (0.02)	7.47 (0.02)	7.50 (0.01)	7.47 (0.02)	7.46 (0.05)	7.51(0.02)*	7.49 (0.02)
Pa_{CO_2} (mmHg)	32.2 (3.3)	33.8 (5.9)	32.0 (3.6)	35.0 (4.5)	37.0 (2.2)	34.6 (3.3)	33.2 (5.5)
Pa_{O_2} (mmHg)	84.4 (4.2)	92.3 (5.9)	81.2 (4.8)	94.2 (7.8)	89.0 (6.7)	83.0 (5.0)	94.4 (11.3)
BE ($mEq \cdot l^{-1}$)	3.1 (2.3)	2.6 (3.8)	3.7 (2.3)	3.3 (1.9)	2.2 (3.2)	4.4 (1.5)	2.1 (4.1)
After the formalin test							
pH	7.50 (0.01)	7.51 (0.04)	7.50 (0.02)	7.49 (0.02)	7.52 (0.02)	7.52 (0.01)	7.53 (0.04)
Pa_{CO_2} (mmHg)	30.8 (2.3)	27.9 (3.8)	31.4 (2.4)	31.5 (3.2)	30.6 (2.7)	31.5 (1.6)	30.9 (6.3)
Pa_{O_2} (mmHg)	83.2 (4.0)	88.7 (8.3)	82.1 (4.5)	86.5 (6.4)	90.1 (6.2)	85.6 (1.4)	88.4 (6.7)
BE ($mEq \cdot l^{-1}$)	3.0 (2.2)	1.3 (2.8)	3.5 (2.0)	2.6 (1.9)	2.0 (1.4)	3.6 (1.0)	2.9 (2.0)

Values are means (SD)
* $P < 0.05$ vs Sham-Shock Yohimbine
Sham-Normo, sham operation-normotensive group; Sham-Shock, sham operation-hemorrhagic shock group; Adrenal-Normo, adrenalectomy-normotensive group; Adrenal-Shock, adrenalectomy-hemorrhagic shock group; Sham-Shock-Y, sham operation-hemorrhagic shock + yohimbine group; Sham-Normo-C, sham operation-normotensive ± corticosterone group; Adrenal-Shock-C, adrenalectomy-hemorrhagic shock + corticosterone group

Table 2. Plasma concentrations of adrenalin, noradrenalin, and corticosterone[a] before and after hemorrhagic shock

Variable	Sham-operated rats	Adrenalectomized rats
n	6	6
Before the hemorrhagic shock		
Adrenalin[a]	455 ± 397	264 ± 271
Noradrenalin[a]	324 ± 81	246 ± 67
Corticosterone[a]	394 ± 106	271 ± 65*
After the hemorrhagic shock		
Adrenalin	12401 ± 4034**	6034 ± 2382*,**
Noradrenalin	2667 ± 931**	1320 ± 326*,**
Corticosterone	519 ± 148**	327 ± 106*

Values are means ± SD
* $P < 0.05$ vs Sham-operated; ** $P < 0.05$ vs before the hemorrhagic shock
[a] Adrenalin and noradrenalin, $pg \cdot ml^{-1}$; corticosterone, $ng \cdot ml^{-1}$

the formalin test. All groups showed a state of respiratory alkalosis, but none showed metabolic acidosis (Table 1). The blood withdrawal volumes in the Sham-Shock, Adrenal-Shock, Sham-Shock-Yohimbine, and Adrenal-Shock-Cor groups were 9.3 ± 2.1 ml, 7.4 ± 0.9 ml, 9.2 ± 1.9 ml, and 9.5 ± 2.1 ml, respectively ($P = 0.14$).

In the 12 rats in which corticosterone and catecholamine levels were measured, the basal catecholamine levels in the unilaterally adrenalectomized group did not differ from those in the sham-operated group. Adrenalin and noradrenalin concentrations increased after hemorrhagic shock. The plasma corticosterone level in the unilaterally adrenalectomized group was significantly lower than that in the sham-operated group before the hemorrhagic shock ($P < 0.05$), and the level did not increase after the hemorrhagic shock (Table 2).

Discussion

In the present study, unilateral adrenal gland destruction attenuated SIA after hemorrhagic shock. Therefore, the hemorrhagic shock-induced analgesia was speculated to be related to the adrenomedullary and/or adrenocortical system. However, the administration of yohimbine (an α2-antagonist) did not increase the pain intensity score in the Sham-Shock group. The hemorrhagic SIA was therefore speculated to be unrelated to the adrenomedullary system. On the other hand, the plasma corticosterone concentration in the adrenalectomized group was significantly lower than that in the sham-operated group before the hemorrhagic shock, and did not increase after the hemorrhagic shock. Furthermore, corticosterone administration reversed the effects of the adrenalectomy on the SIA. Therefore, the present study suggests that the adrenocortical system

takes part in mediating hemorrhagic shock-induced SIA.

Previous studies have indicated that adrenalectomy could influence various types of SIA. Mousa et al. [6] reported that chemical adrenalectomy potentiated analgesia after cold-water swimming stress. In their study, the SIA was reversed by naloxone, which means that endogenous opioids, such as β-endorphin, were an important factor in regulating the SIA. However, our previous study showed that naloxone did not reverse hemorrhagic shock-induced analgesia [5]. Because β-endorphin and adrenocorticotropic hormone (ACTH) are produced from the same precursor [14], we speculated that a high concentration of β-endorphin induced by their chemical adrenalectomy may have been promoted after cold-swim stress and may have potentiated the analgesia. The discrepancy between our results and the results of Mousa et al. [6] might be explained by differences in SIA types (opioid-dependent or opioid-independent). Sutton et al. [9] observed that some SIAs were attenuated by adrenalectomy and that sympathetic blockade failed to reduce the SIA. They pointed out that the basal corticosterone level, rather than that of adrenomedullary substances, was critical to the expression of analgesia. Filaretov et al. [10] showed that SIA and corticosterone concentration changed in parallel in chemically adrenalectomized rats; the SIA phenomenon in their study was observed under a basal condition (stress capable of increasing corticosterone concentration from 100 to 200 $ng \cdot ml^{-1}$), but the SIA disappeared under a condition of suppressed corticosterone secretion (less than 50 $ng \cdot ml^{-1}$). Although the corticosterone concentrations in the study of Filaretov et al. [10] were lower than those in our study, we speculated that these differences were due to their nonsurgical procedure, the bilateral adrenalectomy, and the mild stressor. Corticosterone reactivity to stress also is critical to SIA expression. These conclusions are in accord with our findings.

There were two major methodological differences between the present study and previous studies: the use of the formalin test and the unilateral adrenalectomy. We measured tonic pain using the formalin test because this is closely akin to the clinical situation. The formalin test is a stressful stimulus. However, it was reported that corticosterone did not increase after formalin injection in normal rats [15]. Another investigator reported that formalin stimulus activated the pituitary-adrenocortical system, but that the resulting release of corticosterone did not affect nociceptive processing [16]. Therefore, we think that the stimulation of the formalin test per se did not strongly affect our results. In the present study, we used the pain intensity score of Dubuisson and Dennis [13]. This scoring method has been used in many studies [17], and its validity was evaluated and confirmed by Abott et al. [18] and Coderre et al. [17]. We believe the global responses to the formalin stimulation were evaluated by using this pain score. On the other hand, other studies have investigated the effects of bilateral adrenalectomy [6,7,9,10]. In our preliminary study, we also attempted to destroy the bilateral adrenal glands. However, the bilaterally adrenalectomized rats were severely emaciated and did not lick their hindpaws. Therefore, we used unilaterally adrenalectomized rats.

The mechanisms by which adrenalectomy attenuates SIA after hemorrhagic shock may be affected by adrenocortical hormone levels in peripheral tissue and in the central nervous system. Because corticosterone has an anti-inflammatory effect in peripheral tissue (formalin-injected area in our study), its reduction may have increased nociceptive behaviors in the adrenalectomized group. Corticosterone also modulates the activity of some areas of the central nervous system. Circulating corticosterone was reported to modulate the regulation of norepinephrine or the serotoninergic system in some areas of the brain [19,20]. The presence of glucocorticoid receptors was demonstrated in a large number of various peptidergic neurons [21]. Adrenal steroids alter brain cell function and influence behavioral processes [22]. In regard to pain control, analgesia induced by periaqueductal gray matter stimulation decreased when corticosterone levels were low [23,24]. The low corticosterone level following adrenalectomy may have attenuated SIA via the inactivation of some pain control systems in the brain. Besides adrenocortical hormone, other factors may affect the SIA after hemorrhagic shock. Neuroactive steroids, which are produced by brain tissue [25,26], increased immediately in the blood and brain following stress, and altered pain sensitivity via activation of the γ-aminobutyric acid $(GABA)_A$ receptor [27–29]. Because adrenalectomy has been reported to suppress the stress-induced elevation of neuroactive steroids in plasma and brain [30], the increase in neuroactive steroids and activation of the $GABA_A$ receptor may have been disturbed by the unilateral adrenalectomy in the present study.

In the present study, destruction of the unilateral adrenal gland attenuated the hemorrhagic SIA. The administration of an α2-antagonist (yohimbine) did not affect the SIA observed in the Sham-Shock group. On the other hand, corticosterone administration revived the SIA in the Adrenal-Shock-Cor group. The plasma corticosterone level in the unilaterally adrenalectomized rats was significantly lower than that in the sham-operated rats before the hemorrhagic shock, and did not increase after the hemorrhagic shock. These findings, together with those of our previous study, suggest that the hypophyseal-adrenocortical system, especially the adrenocortical system, plays an important role in hemorrhagic SIA.

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