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The pituitary adenylate cyclase-activating polypeptide (PACAP) protects adrenal function in septic rats administered etomidate

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ABSTRACT

Background: Both hyperinflammation during sepsis and etomidate can suppress adrenal function. In this study, we explored whether treatment with pituitary adenylate cyclase-activating polypeptide (PACAP) relieves adrenal suppression in cecal ligation and puncture (CLP)-induced septic rats.
Materials and methods: Female Sprague–Dawley rats were randomly divided into five groups (n = 7 per group), including the sham group, sepsis group (CLP group), sepsis and etomidate group (CLP + ETO group), PACAP group, and etomidate alone group (ETO group). Rats were sacrificed on the third day of sepsis, and blood and adrenal gland samples were obtained for further testing.
Results: The PACAP reduced the apoptosis rate of adrenal cells and peripheral lymphocytes, improving adrenal function, inhibiting the secretion of interferon gamma (IFN-γ) from peripheral lymphocytes, and slightly relieving the suppression of the adrenal function induced by the injection of etomidate in sepsis.
Conclusion: In septic conditions, the PACAP protects the adrenal gland by regulating peripheral inflammation, which slightly relieves the toxic effects of etomidate on adrenal function.

1. Introduction

Sepsis is the most common causes of death among patients in intensive care units (Du et al., 2013; Hutchins et al., 2014). Approximately 60% of septic patients will eventually develop relative adrenal insufficiency (RAI), which is associated with a high mortality (Annane et al., 2000; Finlay and McKee, 1982). Etomidate, an intravenous general anesthetic agent is used primarily for induction of anesthesia, especially in patients with cardiovascular compromise (Yeung and Zed, 2002). Etomidate inhibits adrenocortical steroid synthesis for up to 24 h after a single injection (de Jong et al., 1984), and may inhibit the necessary initial pro-inflammatory process that occurs during the onset of sepsis (Zhang et al., 2015).

Neuropeptides, with pro- and anti-inflammatory properties, defend the host from inflammation, regulate peripheral organs, and eventually restore homeostasis and a resting state (Brogden et al., 2005; Delgado et al., 1996a; Duan et al., 2014; Reglodi et al., 2015; Sabban et al., 2015). Pituitary adenylate cyclase-activating polypeptide (PACAP), a 38-amino-acid neuropeptide (PACAP38), belongs to the secretin/glucagon/vasoactive intestinal peptide (VIP) family (Miyata et al., 1989), functions as a neurotransmitter and neuromodulator. PACAP and VIP share three types of receptors (PAC1-R, VPAC1-R, and VPAC2-R), and PAC1-R, favors PACAP (Li et al., 2014; Shen et al., 2013). VPAC1-R, and VPAC2-R, expressed in lymphocytes/macrophages, serve as physiological receptors for either PACAP or VIP. PACAP and its receptors expressed throughout the nervous system and peripheral organs, including the adrenal gland and the immune system (Delgado et al., 1998).

This study was designed to examine putative therapeutic effects and mechanisms by which PACAP may contribute to alleviate etomidate-induced adrenal injury in septic rats. Both etomidate and sepsis can induce RAI (Harvey, 2014). PACAP may reduce inflammation during sepsis (Laszlo et al., 2015; Tlili et al., 2015), and adrenal cortex have type I PACAP binding sites (Yon et al., 1993). Thus, we determined whether exogenous PACAP can directly or indirectly reduce adrenal injury in sepsis.

2. Materials and methods

2.1. Animals

Female (8–12 weeks old) Sprague–Dawley rats (Dalian Medical University, China) were used. Animals were housed in the laboratory of the Second Affiliated Hospital of Dalian Medical University. Animals received humane care according to the criteria in the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, and the protocols were approved by the Animal Care and Use Committee of Dalian Medical University. The rats were randomly assigned to 1 of

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5 groups (n = 7 per group, Fig. 1A). To mimic a clinical situation, 6 h after cecal ligation and puncture (CLP), rats in the CLP + ETO group were treated with a single dose (2 mg/kg) of etomidate (Enhua Pharmaceutical, China) intravenously. To assess the effects of the PACAP, the PACAP group was treated intravenously with a single dose (50 nmol/rat) of PACAP (PACAP 38, Alpha Diagnostic Intl, San Antonio, Texas) immediately after etomidate treatment. The ETO group was treated with a single dose of etomidate without CLP. As a positive control, the CLP group only underwent CLP. The sham group underwent pseudo-CLP surgery, which is the same procedure as CLP but without CLP.

2.2. Experimental protocol

All surgical procedures were performed in aseptic conditions, and the animals were anesthetized with continuous 2–4% sevoflurane using a small animal anesthesia machine (Raymain, Shanghai, China) during the surgery. The right jugular veins were cannulated with catheters as described in the literature (Hughey et al., 2011). Incisions were created on the backs of the rats, and the catheters were exteriorized on the backs of the animals. Drugs were administered via the venous catheters; blood samples were collected via the catheters with minimal stress to the animals. Three to five days after surgical catheterization, CLP was performed as described by Wichterman et al. (Wichterman et al., 1980). During the CLP operation, one-third of the distal cecum was ligated with 4–0 silk (Ethicon, Shanghai, China) below the ileocecal valve and was penetrated twice with a 21-gauge needle. For postoperative analgesia, 1 ml saline with 0.25 mcg/mL sufentanil (Yichang Renfu Pharmaceutical, China) and 0.05 mg/mL midazolam (Enhua Pharmaceutical, China) was administered subcutaneously. Blood samples for adrenocorticotropic hormone (ACTH) stimulation test were collected on the third day of the CLP surgery, which was the early phase of sepsis with excessive inflammation. The rats were then euthanized to remove the bilateral adrenal glands.

2.3. Corticosterone response to exogenous ACTH

The plasma corticosterone response to exogenous ACTH (Sigma-Aldrich, St. Louis, MO) was determined as described previously (Carlson et al., 2006). First, dexamethasone (0.25 mg/kg; Sigma Aldrich, St. Louis, MO) was infused intravenously at 15:00 PM. Two hours later, rat ACTH (10 ng/kg) was infused. Blood samples of 0.5 ml were obtained immediately at 30 min after ACTH administration (Fig. 1B). Each sample was replaced with an equal volume of saline-containing heparin (100 units/mL).

Serum corticosterone was measured with rat-specific, enzyme-linked immunosorbent assay kits (Cayman Chemical, Ann Arbor, MI) according to the manufacturer's instructions. This kit was characterized by a broad working range and the specificity was 100% for corticosterone, 0.18% for cortisol, and 0.02% for cortisone.

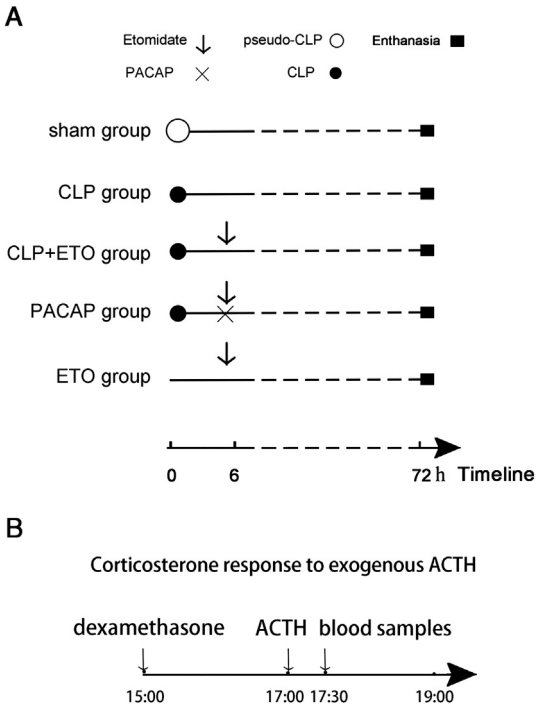

Fig. 1. Schematic diagram of the etomidate/PACAP administration protocol for each group (A) and ACTH stimulation test (B) in each group.

2.4. Flow cytometry

After removing the adipose tissue and adrenal medulla, the fresh rat adrenal glands were cut into small pieces and washed three times in DMEM/F12 medium (Gibco, Life Technologies, Carlsbad, CA). Dispersed cells were obtained by digestion in DMEM/F12 medium with 1 mg/ml collagenase I (Amresco, Solon, OH) for 30 min. The cell suspension was filtered through a 75-mm nylon gauze and washed by centrifugation at 800 rpm for 8 min. Lymphocytes were separated through density gradient by mouse lymphocyte separation medium (Haoyang, Tianjin, China) in blood sample (2 ml) collected via the venous catheters using heparinized tubes. Cells of the adrenal gland and lymphocyte were prepared as a single-cell suspension (between 1×106 and 8×106 cells/ml) and stained with various antibodies from Biolegend (San Diego, CA) for 30 mins to 1 h in an Fc block on ice. Intracellular cytokine staining was performed with the Foxp3 intranuclear staining kit from Biolegend, as recommended by the manufacturer. A fluorescein isothiocyanate (FITC)-labeled annexin V and propidium iodide staining kit (Biouniquer, Nanjing, China) was used to stain the primary adrenal cells and lymphocyte. After fixing and blocking, the cells were immunolabeled with PE conjugated rabbit anti mouse antibodies specific to CD4/CD8 (Biolegend, San Diego, CA), and FITC conjugated rabbit anti mouse antibodies specific to IFN-γ, LPS-specific toll-like receptor 4 (TLR-4) (Biolegend, San Diego, CA). A flow cytometer (FACSCalibur; BD Biosciences, CA) was used to assess the apoptosis rate, TRL-4 expression, and cytokine secretion.

2.5. Statistical analysis

For comparisons among the groups, one-way analysis of variance followed by a *post hoc* Tukey's test, when appropriate, was used for comparisons among the groups. All data were analyzed with GraphPad Prism (version 6.02, GraphPad Software, CA). P-value < 0.05 was considered significant. The quantitative data are presented as the mean ± standard error of the mean.

3. Results

3.1. The effects of PACAP on adrenal gland apoptosis

To explore the effect of the PACAP on the adrenal gland, the early apoptosis rate of adrenal glands were measured in each group. We analyzed the apoptosis rate by dual staining with Annexin V-FITC and PI. As is shown in Fig. 2A, adrenal apoptosis in septic rats increased significantly in the CLP group compared with the sham group. Etomidate treatment exacerbates the adrenal apoptosis (group CLP + ETO) markedly. PACAP treatment relieves sepsis/etomidate-induced apoptosis in the adrenal cells. These results demonstrate that the PACAP group exhibited reduced apoptosis rates compared with the CLP + ETO group, thus suggesting the protective effect of PACAP on adrenal apoptosis in septic rats administered etomidate.

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